БЮЛЛЕТЕНЬ
ЭКСПЕРИМЕНТАЛЬНОЙ
БИОЛОГИИ
И МЕДИЦИНЫ

Bulletin of
EXPERIMENTAL BIOLOGY AND MEDICINE

Supplement to No. 1

1957

IN ENGLISH TRANSLATION

CONSULTANTS BUREAU, INC.

227 WEST 17TH STREET NEW YORK 11, N. Y.

an agency for the interpretation of international knowledge

BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE

(BYULLETEN EKSPERIMENTALNOI BIOLOGY I MEDITSINY)

Supplement to No. 1

1957

Editorial Board

V. N. Chernigovsky (Editor)

A. Ya. Alymov, N. A. Kraevsky, G. N. Kryzhanovsky (Secretary)

A. V. Lebedinsky (Acting Editor), I. N. Maisky, V. A. Negovsky, V. A. Sanotsky,

L. G. Trofimov, M. A. Vorontsova, O. E. Vyasov, N. A. Yudaev, G. N. Silov

A Publication of the Academy of Medical Sciences of the USSR

IN ENGLISH TRANSLATION

Copyright, 1958

CONSULTANTS BUREAU, INC.

227 West 17th Street

New York 11, N. Y.

The National Institutes of Health of the Public Health Service has supported in part the cost of publishing BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE through the purchase of copies for distribution to American medical libraries.

Printed in the United States

Annual Subscription	$20.00
Single issue	10.00

Note: The sale of photostatic copies of any portion of this copyright translation is expressly prohibited by the copyright owners. A complete copy of any paper in this issue may be purchased from the publisher for $7.50.

0-30

STUDY OF CORONARY BLOOD FLOW IN UNANESTHETIZED DOGS

M. E. Marshak and G. N. Aronova

From the Laboratory for the Physiology and Pathology of Respiration and Circulation (Director: Honored Scientific Worker Prof. M. E. Marshak), Institute of Normal and Pathological Physiology (Director: Prof. V. N. Chernigovsky, Member Acad. Med. Sci. USSR), Acad. Med. Sci. USSR.

(Received September 12, 1955. Presented by V. N Chernigovsky, Member Acad. Med. Sci. USSR)

Data relating to the physiology and pathology of the circulation of the heart have chiefly been derived from short experiments on animals. Essex alone was able, using a thermoelectric method, to observe changes in the rate of flow of blood in the coronary arteries of dogs, kept under natural conditions over periods of from some days up to a maximum of two weeks after the operation [2].

We have recorded the dynamics of changes in flow-rate in a branch of the coronary artery of unanesthetized dogs in response to various stimuli, over a period of six weeks after the operation.

EXPERIMENTAL METHODS

Noyens' thermoelectric method [3] was applied to the study of variations in the rate of flow of blood in intact vessels, modifying the thermoelectrodes in order to apply them to the conditions of a long experiment. The thermoelectrode used for implantation into the coronary vessels differed from that described by Noyens for short experiments, as described by M. E. Marshak and M. M. Voll [1] in its smaller dimensions (5-6 mm wide) and in its low weight (about 1.3 g). A nickeline strip was used for warming the junction of the thermocouple. We endeavored to exclude constriction of the artery, and to ensure prolonged functioning of the thermoelectrode. The thermoelectrode was applied to an encircling branch of the left coronary artery of the experimental animals. The operational technique differed from that used by Essex, who sutured the leads from the thermoelectrode to the myocardium, which is not without effect on the functioning of the heart and on its blood supply. We sutured the leads from the electrode to the inner surface of the thoracic wall. The terminals of the leads were exteriorized at the neck, where they were connected during experiments with leads from the galvanometer and accumulator.

EXPERIMENTAL RESULTS

Changes characteristic of disturbance of coronary circulation were seen in the T wave of the electrocardiogram during the first few days after the operation. Full restoration of coronary circulation took place on the 7th day, as seen from the electrocardiogram.

Only insignificant fluctuations in the rate of flow of blood in the coronary artery of dogs standing at rest on the bench were observed. Feeding (milk or meat) caused a well-defined rise in the flow-rate, persisting for several minutes after completion of feeding as is shown by Figure 1.

Inhalation of air of a raised CO_2 content (about 2-3%) raised the rate of flow in the cornary artery, and substitution of ordinary air led to a slow return to the initial value.

As for short experiments, distention of the rectum of unanesthetized dogs for 1 minute caused a marked increase in the blood supply to the heart. As appears from Figure 1, b, the heightened flow rate persisted for 4 minutes after distention had been discontinued, and return to the initial value took place only very slowly.

The effect on the coronary flow rate of static loading of the dogs (a saddle supporting a weight of 5-8 kg was fastened to their back) was also investigated. A well-defined rise in the rate of flow was registered immediately after loading, and the raised level persisted as long as the load remained, and for some minutes after its removal. Restoration of the normal rhythm and depth of respiration took place much sooner than restoration of the initial flow rate. Repetition of the experiment, with the same load, led to a smaller elevation of flow-rate; this can be regarded as a training effect.

Figure 1,c shows the effect on the rate of flow in the coronary artery of an aggressive stimulus, in this case the appearance of a cat. As is seen from the curve, the flow-rate rose gradually to a maximum when the dog was most excited, and displayed abrupt fluctuations, with heightened respiration.

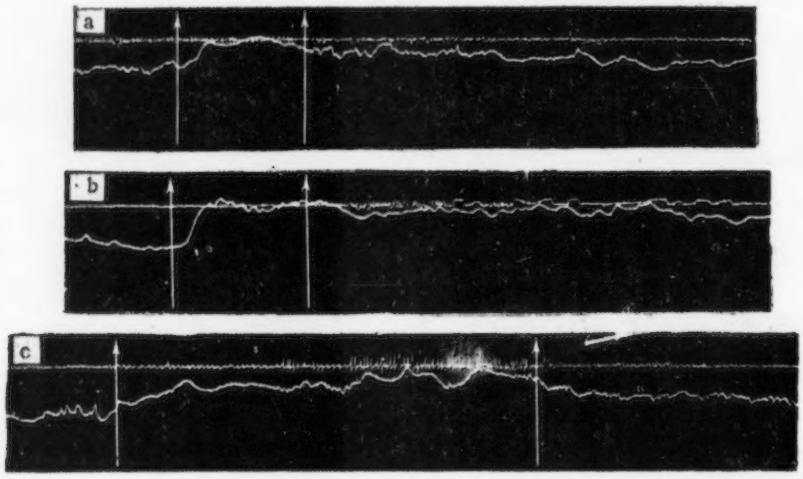


Fig. 1. Changes in the rate of flow in coronary arteries of dogs.
a) Feeding; b) distention of the rectum; c) display of a cat. Explanation of curves (from above down): respiration, rate of flow of blood. The arrows indicate commencement and termination of stimulus.

The effects of painful electrical stimulation of a hind leg on the rate of flow in a coronary artery are shown in Figure 2.

Fig. 2. Effect on coronary blood flow of electrical stimulation of a leg.
Explanation of curves as for Figure 1. The arrows ↑↑ indicate duration of painful stimulation, and the arrow ↓ indicates the moment of fall of the instrument, which frightened the dog.

A transient fall in flow-rate, followed immediately by a rise, was observed at the beginning of the period of painful stimulation. This initial fall in flow-rate was a regular feature of these experiments. It is highly characteristic of the effect of painful stimulation that whereas the effect of the stimuli described above was undirectional, leading to rise in flow-rate only, the rate of supply of blood to the heart diminished only at the moment of application of painful stimuli. This phenomenon was observed whenever the experiment was repeated, whether on the same day or on different days.

The diminution in the supply of blood to the heart at the moment of initiation of painful stimulation is evidently due to its powerful, violent, and abrupt action on the nervous system. This view is supported by the following observations. During one of the experiments on painful stimulation a dog standing on the bench began to struggle after a fairly strong current had been switched on, and knocked over one of the instruments, which fell to the floor with a loud clatter. This intensified the emotional state of the dog. In this, as in all other experiments of this type, a brief fall in flow-rate was observed immediately after initiation of the painful stimulus,

followed by a rise, but it is noteworthy that after the fall of the instrument the rate of flow fell rapidly to below the initial level, as is shown in Figure 2. During another experimenton the effect of static loading the harness carrying the load accidentally slipped forward and downward, leading to an unexpected compression of the chest, which frightened the dog, causing it to struggle. Here,too, we observed a marked fall in the rate of flow of blood.

The effects on the rate of flow in the coronary artery of painful stimuli and of feeding remained the same over the whole period of observation following the operation.

One dog with an indwelling thermoelectrode died on the 42nd day after operation. Up to the day of its death the dog showed no external symptoms or signs of ill-health. Marked changes in the electrocardiogram were, however, seen two days before it died.

The above data illustrate the importance of the study of the physiology and pathology of the coronary circulation in unanesthetized animals. Under such conditions it is possible to follow changes in the blood supply of the heart due to various environmental factors, such as feeding, muscular exertion, etc.; such observations would not be possible under the conditions of a short experiment. Moreover, the experiments on painful stimuli gave clear evidence of the effect of the functional state of the central nervous system on the reaction of the coronary arteries during the experiment.

LITERATURE CITED

[1] M. E. Marshak and M. M Voll, Arkh. Biol. Nauk SSSR, 63, No. 3, 40-48 (1941).

[2] H. E. Essex, J. F. Herrick, E. J. Baldes and F. C. Mann, Am. J. Physiol., 1936, Vol. 117, No. 2, pp. 271-279.

[3] A. K. Noyons, M. von Westenrijk and J. Jongbloed, Arch. neerl. de physiol., 1936, Vol. 21, pp. 377-432.

REFLEX EFFECTS ON VASCULAR TONUS FROM CARDIAC MECHANORECEPTORS

N. I. Lukshina and A. M. Ugolev

From the Chair of Normal Physiology (Director: Prof. A. D. Slonim), Kalinin Medical Institute (Director: Prof. R I. Gavrilov)

(Received June 24, 1955. Presented by V. N. Chernigovsky, Member Acad. Med. Sci. USSR)

One of the first indications of the existence of receptor functions of the myocardium and endocardium emerged from the so-called Bezold-Jarisch effect.

Bezold and Hirt [4] found in 1867 that injections of veratrine slowed the action of the heart. They interpreted this effect as being a cardiocardiac reflex. Similar effects were described by Jarisch and Henze [6] and by Richter and Schrockschnadel [9]. According to Jarisch, this reaction is effected through cardiac mechanoreceptors, the sensitivity of which is raised by veratrine; the effect involves stimulation of the vagus, with inhibition of sympathetic nerves.

Schaeffer [10] and others showed later that volleys of impulses from cardiac branches of the vagus are as a rule synchronous with contraction of the heart, but under the influence of veratrine the impulses become continous (Jarisch and Zotterman [7]). The action of veratrine is exerted on receptors situated in the left ventricle (Schaeffer [10]).

A considerable number of papers have been published over the last few years, dealing with the study of the mechanisms of the nervous pathways of the Bezold-Jarisch reflex (see the detailed review by Dawes and Comroe [5]).

In his recent publications V. V. Frolkis [1] has shown that the activity of the heart involves the stimulation of myocardial receptors, and this significantly affects the excitability of the central nervous system.

This author showed in a second paper that during stimulation of a peripheral section of the vagus, disturbance in the rhythmicity of the heart caused stimulation of its interoceptive apparatus. This initiates the compensatory reaction, known as the phenomenon of "evasion" of vagus control by the heart [2].

Finally, Frolkis states in his last paper [3] that in experimental myocarditis the excitability of nerve centers is heightened, and that reflexes affecting the heart proceed more intensively even when the strengh of the stimulus is smaller.

However, in the majority of the above-cited papers the effects of factors exerted through the blood stream, and of reflexogenic vascular zones, were not fully excluded.

We have in the present research attempted to study the effects of reflex reactions originating in the heart on the blood vessels. In undertaking this research we were motivated both by theoretical concepts of the functional unity of the cardiovascular system and by clinical observations which gave evidence that stimulation of parts of the sensory apparatus of the heart affects the state of the vascular bed. In our experiments we excluded all possible connections between the heart and the blood vessels, other than nervous ones, and we applied adequate stimulators.

EXPERIMENTAL METHODS

In order to exclude all except reflex reactions between the heart and the vessels they were perfused separately, after being isolated (Figure 1).

A frog with an intact central nervous system was fixed rigidly to a special table. Both aortas were ligated, the right one closer to the heart, and the left one further from it. A Straub cannula was introduced into the centricle through the left aorta, below the ligature. The vessels draining into the venous sinus and the pulmonary arteries were ligated, taking care not to damage cardiac twigs of the vagus nerve.

Perfusion of the vessels was achieved through the right aortal arch, above the ligature isolating it from the heart. The Ringer solution was introduced into the vessels at constant pressure. The tonus of the vessels was assessed from the number of drops of perfusate emerging from the anterior abdominal vein over a period of 30 seconds.

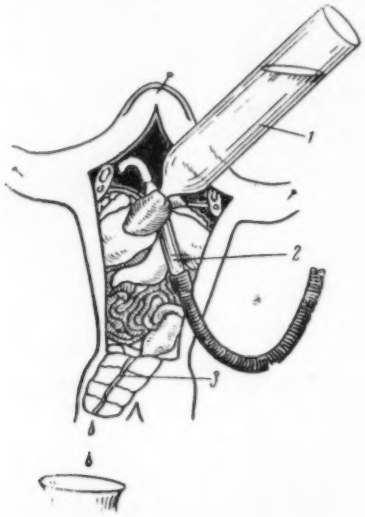


Fig. 1. Schematic representation of separate perfusion of the isolated heart and vascular system of a frog.
1) Straub cannula, introduced through the left aorta into a ventricle; 2) perfusing cannula inserted into the distal section of the severed right aorta, serving for the continuous perfusion of the vascular system with saline under constant pressure; 3) anterior abdominal vein.

Under these conditions changes in cardiac acitivity could not affect the vessels as a result of variations in the rate of flow of the fluid.

We had previously found that in the elucidation of the normal reflex interrelations between different parts of the cardiovascular system it is essential that adequate stimuli should be applied.

In our experiments stimulation was effected by changing the hydrostatic pressure in the Straub perfusion cannula. We placed 0.2 ml of Ringer solution in the cannula, and then added more solution up to 3 ml, thus raising intracardiac pressure by 15-25 mm of water. After a certain time the solution was sucked off down to the initial volume. The pressure was varied several times during each experiment, and in every case the number of drops emerging was registered.

Experiments on change in hydrostatic pressure in the right auricle, and in all parts of the heart simultaneously, were performed in an analogous way. Distention of the isolated auricle was achieved by introducing the cannula in one of two ways: a) through the anterior vena cava, and b) through a small aperture in the anterior wall of the ventricle.

EXPERIMENTAL RESULTS

After total isolation of the heart from the vascular system, raising of the hydrostatic pressure within a ventricle caused a diminution in the rate of flow of perfusate from the anterior abdominal vein, as compared with the initial rate. This is evidence of reflex raising of the tonus of the vessels. This effect was observed in 51 experiments, of a total of 55 in this series:

in 3	experiments	the	flow	fell	by	from	50 to 75 %
" 29	"	"	"	"	"	"	20 to 30 %
" 8	"	"	"	"	"	"	10 to 20 %
" 9	"	"	"	"	"	"	5 to 10 %

There was no change in rate of flow in 4 experiments.

We also observed a fall in the rate of flow of perfusate in 12 of 15 experiments involving raised hydrostatic pressure in the right auricle:

in 5	experiments	the	flow	fell	by	from	25 to 35 %
" 3	"	"	"	"	"	"	10 to 20 %
" 4	"	"	"	"	"	"	5 to 10 %

No change was found in 3 experiments.

Finally, raising the hydrostatic pressure in all the divisions of the heart caused a particularly large fall in the rate of flow of perfusate, in all the 14 experiments performed:

in 1	experiment	the	flow	fell	by	80 %
" 3	experiments	"	"	"	"	from 60 to 75%
" 9	"	"	"	"	"	40 to 50%
" 1	experiment	"	"	"	"	25 %

It should be noted that when all the chambers of the heart were distended simultaneously the tonus of the vessels rose very considerably.

Two cases are represented in Figure 2: a) change in flow rate when all parts of the heart are distended simultaneously, and b) when ventricle alone is distended.

It is evident that the effect is much greater in the former case than in the latter. The nature of the reaction is such as to preclude the possiblity of artifacts. Were the heart not completely isolated from the vessels the effect of elevation of pressure in the former would presumably be that a certain amount of saline would find its way from the heart to the vessels, and this should augment the flow from the anterior abdominal vein. This was shown to be the case in special experiments performed by us, in which ligation of the vessels was

incomplete. In these experiments a certain amount of solution passed from the vessels to the heart, raising the level in the Straub cannula. The possibility of incomplete isolation of the vascular system, or of the intervention of any factors other than reflex ones, are excluded by the observation that the level remains constant in the Straub cannula, as well as by the nature of the reaction.

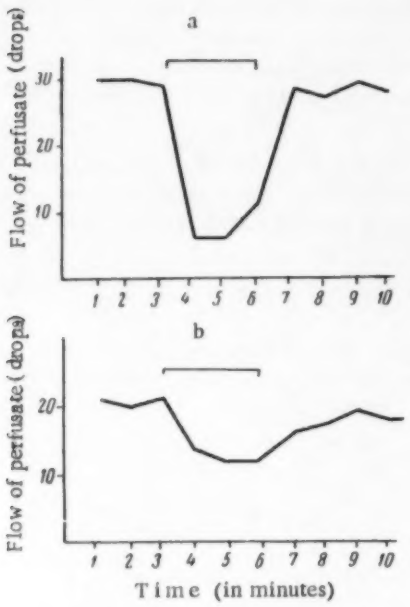


Fig. 2. Reflex changes in vascular tonus following raising of intracardiac pressure, under conditions of isolation of the heart from the vessles. a) simultaneous distention of all parts of the heart; disten ion of an isolated ventricle alone; ⌜⌝ — pressure raised.

Our experiments show that the sensory structures of the heart are able to react to variations in pressure within its chambers. It is most probable that the receptors are sensitive not to pressure itself, but to distention of the muscular walls of the heart.

It may be concluded from our experiments that the most typical reaction to distention of the heart is raising of vascular tonus.

Although the conditions of our experiments excluded the possibility of any factors whatsoever other than reflex ones, we performed two series of experiments designed to confirm the reflex nature of the effects observed by us.

In the first series we destroyed the central nervous system of the frogs 30-45 minutes before the experiment, and then varied intracardiac pressure while perfusing the vascular system. In all the ten experiments of this series vascular tonus either remained steady, or else rose gradually and smoothly throughout the experiment, without any relation to variations in hydrostatic pressure within the heart.

In the second series of experiments we studied effects originating in the heart and acting on the vessels, under conditions of isolated perfusion, as before, but before and after section of the vagosympathetic pathways. In 19 of 25 experiments the tonus of the vessels diminished immediately after cutting the nerves, and the outflow of perfusate increased:

in	3 experiments	by	from 40 to 50 %
"	1 experiment	"	30 %
"	10 experiments	"	from 10 to 20 %
"	5 "	"	" 5 to 10 %

There was no change in rate of flow in 4 experiments, and a diminution of 5-8% in 2 experiments.

Vascular tonus then returned to the initial level in all 25 experiments, and was not thereafter affected by varying intracardiac pressure (Figure 3).

It follows that the afferent pathways of the given cardiovascular reflex are contained in the vagosympathetic trunks. Interruption of the stream of impulses from the cardiac mechanoreceptors when thenerve trunks are severed leads in most cases to a lowering of the tonus of the vessels, i.e., the effect is the opposite of that observed when intracardiac pressure is raised. However, the nature of the effect produced by intracardial mechanoreceptors on vascular topus is somewhat unexpected, and merits special consideration.

Enlargement of the heart under conditions of enhanced venous flow leads to a rise in arterial pressure, based on Starling's law, Bainbridge's reflex, and MacDowell's reflex. It may be supposed that under these conditions reflexes originating from cardiac mechanoreceptors should bring about a compensatory reaction, lowering excessive increase in arterial pressure. We, however, found a somewhat different effect, viz., to the raising of arterial pressure due to intensification of the action of the heart was superadded the effect due to increase in arterial tonus.

There is scarcely any need to point out that, as far as maintenance of constancy of arterial pressure is concerned, this reflex is pointless, and is in conflict with our basic concepts regarding these matters. One can only think that the rise in blood pressure associated with increased venous return, and proceeding in accordance with Starling's law and Bainbridge's reflex, is to some extent dependent on reflexes emanating from cardiac mechanoreceptors affecting vascular tonus, as described above.

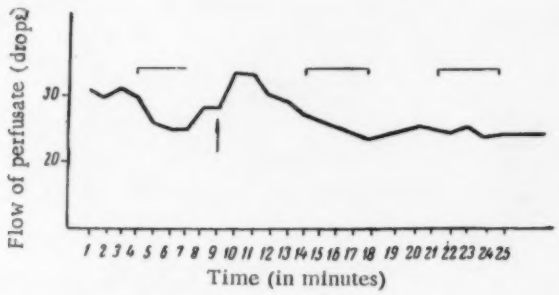


Fig. 3. Effect of section of the vagosympathetic trunks, under conditions of isolated perfusion of the heart and vessels, on reflexes from cardiac mechanoreceptors ⌐¬ — pressure raised, ↑ vagotomy.

A consideration in greater detail of the conditions governing drainage of the venous system may assist in the comprehension of the role of the above-described reflexes, in the system of reactions regulating circulation of the blood. Accumulation of blood in the venous system must inevitably raise pressure within the veins, and distend their walls. The latter effect is associated, as was long ago shown by Bainbridge, with a sympathetic reaction from the heart, and, consequently, with intensification of transfer of blood from the veins to the arteries. Rise in venous pressure stimulates the work of the heart in other ways, first, according to Starling's law, and secondly by MacDowell's reflex. It thus appears that at least three different mechanisms are implicated in the regulation of transfer of blood from the venous to the arterial bed.

On closer consideration, however, redistribution of the blood between the venous and arterial systems cannot be thought to be a consequence of increase in cardiac output alone, since the latter leads not only to transfer of additional amounts of blood from the large veins to the large arteries, but also to acceleration of its flow from the arterioles through the capillaries to the venules, if the resistance of the vessels to the flow of blood remains unchanged. Discharge of blood from the venous system can only be effective if the enhanced flow of blood from the veins to the arteries (the cardiac component of the unloading reaction) is coupled with impedance of its return from the arteries to the veins (vascular component of the unloading reaction).

It may be thought that the reflex observed by us contributes to the achievement of this requirement.

LITERATURE CITED

[1] V. V. Frolkis, Byull. Eksptl. Biol. i Med., No. 4, 45-47 (1952).

[2] V. V. Frolkis, Byull. Eksptl. Biol. i Med., No. 10, 19-22 (1952).

[3] V. V. Frolkis, Byull. Eksptl. Biol. i Med., 37, No. 3, 21-24 (1954).

[4] A. Bezold, Untersuchungen d. physiol. Lab. in Wurzburg, 1867, No. 1, S. 181-194.

[5] G. S. Dawes and J. H. Comrde, Physiol. Rev., 1954, Vol. 34, No. 2, pp. 167-201.

[6] A. Jarisch and C. Henze, Arch. f. exptl. Pathol. Pharmakol., 1937, Bd. 187, S. 706-730.

[7] A. Jarisch and J. Zotterman, Acta Physiol. Scandinav, 1948, Vol. 16, pp. 31-51.

[8] J. V. Jones, Brit. J. Pharmakol., 1953, Vol. 8, pp. 352-355.

[9] H. Richter and H. Schrockschnadel, Arch. f. exptl. Pathol. Pharmakol., 1938, Bd. 191, S. 23-29.

[10] H. Schaeffer, Ergebn. d. Physiol., 1950, Bd. 46, s. 71.

SIGNIFICANCE OF THE REFLEX FROM THE SUPERIOR VENA CAVA IN THE REGULATION OF BLOOD AND LYMPH CIRCULATION

V. V. Petrovsky

From the Chair of Normal Physiology (Director: Prof. V. V. Petrovsky, Bashkir Medical Institute, Ufa

(Received March 16, 1956. Presented by V. N. Chernigovsky, Member Acad. Med. Sci. USSR)

The findings of a number of members of our Department (Z. G. Valeeva, D. I. Smirnov, K. V. Kovanov, G. H. Kotova), relating to functional connections between the lymphatic and blood vessels, have been presented in an earlier paper [1].

In the present paper we present some data obtained from further investigation and study of the significance of the reflex from the superior vena cava in the regulation of blood and lymph circulation.

We measured the pressure of lymph in the thoracic duct of a dog, using a mercury manometer, with simultaneous registration of arterial and venous (external jugular vein) pressure, with the object of ascertaining the strength of reflex contraction of the lymphatic vessels. In some cases we perfused the cervical lymphatic duct. Our experiments showed that intravenous injection of large volumes of physiological saline raises arterial pressure by 5-40 mm Hg, and venous pressure from 0-2 to 8-12 cm of water; at the same time the cervical lymphatic duct contracted to a state of complete spasm, and the pressure in the thoracic duct rose to 40-80 mm Hg (Figure 1).

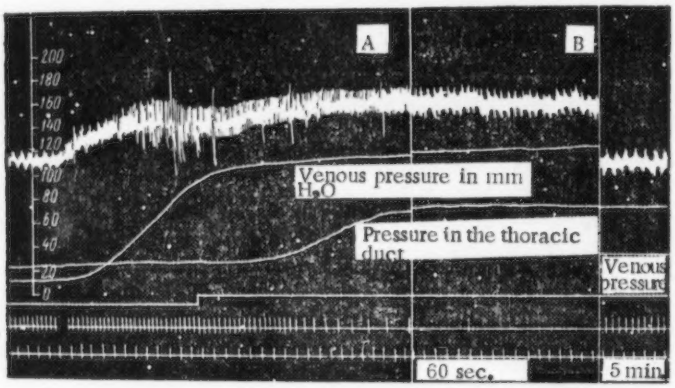


Fig. 1. Effect of intravenous injection of 400 ml of physiological saline on arterial and venous pressure and, pressure in the thoracic lymph duct, and on the tonus of the cervical lymph duct. A) Kymograph stopped for 1 minute; B) kymograph stopped for 6 minutes. Explanation of tracings, from above down: blood pressure in the carotid artery; lymph pressure in the thoracic duct, in mm Hg; venous pressure (right external jugular vein), in mm of water; flow of fluid through the cervical lymphatic duct (number of drops); time marker (5 seconds).

It might be supposed that thoracic duct pressure rose as a consequence of passive distention, due to rise in capillary pressure. In order to check this possibility we measured thoracic duct pressure during introduction of adrenaline. As is evident from Figure 2, the pressure in the thoracic duct, initially 40 mm Hg, rose rapidly after injection of adrenaline to a valueof 75 mm. The rises in lymphatic duct and blood pressure took place simultaneously. The maximum rise in blood pressure also coincided in time with that of lymph. Venous pressure, which was measured separately in this experiment, was 0 cm of water before injection of adrenaline, and rose to 12 cm of water during its action. Since the rise in arterial pressure was synchronous with that in the thoracic duct, it is unlikely that the latter could have been due to intensification of lymph formation, which could have led to over-filling of the lymphatic vessels with lymph, and hence to a rise in lymphatic pressure.

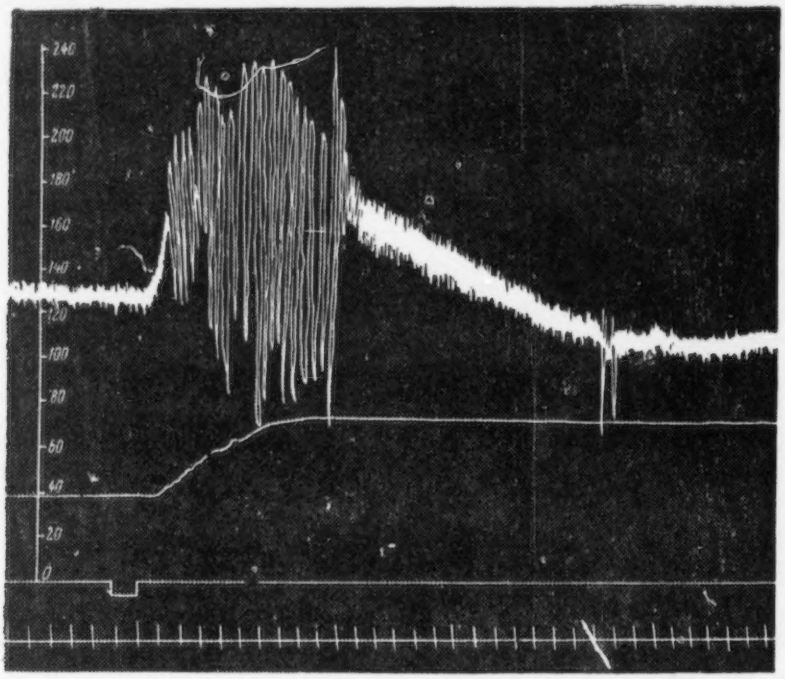


Fig. 2. Effect of intravenous adrenaline (0.000015) on blood pressure and pressure in the thoracic duct.
Explanation of tracings, from above down: blood pressure in the carotid artery: pressure in the thoracic duct, in mm Hg; signal showing time of introduction of adrenaline; time marker (5 seconds).

In a second series of experiments we measured the pressure in the cisterna chyli, into which drain a number of lymph ducts draining the viscera. We raised venous pressure by injection of physiological saline and adrenaline, and in this way raised the pressure in the cisterna chyli to over 60 mm Hg.

The results of our experiments provide evidence of the absence of lymphatic-venous anastomoses; if these existed such rises in thoracic duct pressure as we registered could not have been possible.

It may be supposed that, under conditions of normal blood circulation with raised venous blood pressure, causing increased resistance to the flow of lymph, a reflex contraction of the lymphatic vessels takes place, leading to expulsion of part of their contents into the blood vessels. This causes an increase in the volume of circulating blood. This need not necessarily lead to still further increase in venous pressure, and hence to still further constriction of the lymph ducts. As is known, rise in venous pressure gives rise to a reflex acting on the heart (the Bainbridge reflex). The heart beat becomes more frequent, the minute volume rises, and venous pressure falls. In correspondence with the increased blood volume the capacity of the vessels of the systematic

circulation rises, in response to stimulation from the reflexogenic zones of the carotids and aorta. This explains the significance of the reflex from the superior vena cava under conditions of normal activity of the cardiovascular system.

Venous pressure also rises when the activity of the heart is depressed, but the organism is not able to counteract this tendency by reflex stimulation of cardiac activity. Raised pressure in the superior vena cava evokes a pressor reflex acting on the lymphatic vessels, and impeding the flow of lymph. Lymph is at first retained in the parts of the body most distant from the heart, and then, with increasing venous pressure and striction of lymphatic vessels, edema makes its appearance in other parts of the body. Hulse [3] has reported that in patients displaying progressive edema the thoracic duct and the cisterna chyli are empty, but fill up with lymph as soon as resorption of edema fluid begins. According to Hulse, the incidence of this effect is so regular that it is possible to deduce from it what stage of the edematous process is present, without clinical examination of the patient.

This reasoning applies, obviously, only to edema associated with raised venous pressure. Thus factors tending to lower venous pressure will bring about a weakening of the pressor reflex, and so favor transfer of edema fluid to the blood stream.

Rusnyak [2] has in a recently published paper drawn attention to certain inconsistencies with Starling's theory of causation of edema, such as, for example, the beneficial effect of ligating the inferior vena caca of patients with cardiac decompensation. Not only does this not aggravate the edema of the lower extremities, as would be expected from Starling's theory, but on the contrary, it lessens it.

Rusnyak does not enter into a consideration of the reasons for this effect. We would, on the basis of our experimental findings, suggest the following explanation. Ligature of the inferior vena cava involves a diminution in the flow of blood to the right auricle, at least until the establishment of an adequate collateral circulation. As a result, the pressure in the right auricle and the superior vena cava falls, and the pressor reflex becomes weaker. In addition to this, the pressure in the part of the vein below the ligature rises, causing reflex dilatation of the lymphatic channels, which will tend still further to inhibit the pressor reflex from the superior vena cava. In consequence, the patency of the lymphatic vessels is restored, and the edema of the lower extremities disappears. We would emphasize that Rusnyak also attaches significance to the lymphatic vessels in development of edema.

LITERATURE CITED

[1] V. V. Petrovsky, Fiziol. Zhur. SSSR, No. 3 (1954).

[2] I. Rusnyak, Klin. Med. No. 1, 23-35 (1954).

[3] W. Hulse, Klin. Wschr., 1923, No. 2, S. 63-65.

INFLUENCE OF SPLENIC INTEROCEPTORS ON BLOOD PRESSURE AND RESPIRATION IN ONTOGENESIS

L. A. Palgova

From the Chair of Normal Physiology (Director: Prof. A. P. Polosukhin), Kazakh Medical Institute, Alma-Ata

(Received November 18, 1955. Presented by V. N. Chernigovsky, Member Acad. Med. Sci. USSR)

We know from the literature [6] that the spleen of adult animals (dogs, cats) contains receptors, which when stimulated evoke reflex changes in respiration, arterial and venous pressure, and flow of lymph.

The present paper is devoted to a study of the effects of splenic interoceptors on blood pressure and respiration in ontogenesis.

EXPERIMENTAL METHODS

We performed short experiments on the stimulation of splenic receptors of puppies aged from 1 day to 3-4 months.

We studied reflexes affecting respiration and arterial pressure, originating from splenic baroceptors and chemoceptors. The baroceptors were stimulated by blocking the venous outflow from the spleen, thus causing engorgement with arterial blood, or by raising the pressure of fluid with which the spleen was being perfused through its blood vessels. The spleen was in the latter case in a state of humoral isolation. Perfusion was performed with Ringer-Locke solution. The chemoceptors were stimulated by adding histamine and acetylcholine to the perfusion fluid.

We measured the blood pressure in the carotid artery during the experiments, using a Ludwig mercury manometer. Respiration was recorded through a tracheal cannula connected with a Marey capsule. We used 52 puppies in our experiments.

EXPERIMENTAL RESULTS

We performed 3 series of experiments with the object of elucidating the effects of splenic receptors on blood pressure and respiration at different ages. In the first 2 series we examined the origination of interoceptive effects from splenic baroceptors on blood pressure and respiration. For this purpose we raised the blood content of the spleen by blocking venous outflow (1st series of experiments).

The main trunk of the splenic vein was exposed, and a ligature was placed beneath it, in readiness for tightening. Arteries and nerves accompanying the vein were preserved intact. All other vessels were ligated and severed. The ligature under the splenic vein was then tightened, thus temporarily preventing the outflow of blood from the spleen, while retaining its arterial supply.

In this way we achieved an engorgement of the spleen with blood, as could be seen by direct observation; its volume increased, and it acquired a firmer consistency. In all the experiments of this series (28), using puppies aged up to $1^1/_2$-2 months, the systemic arterial pressure fell. The changes in blood pressure took place immediately after constricting the vein, i.e., with an extraordinarily brief latent period. Apart from this, we observed a prolonged after-effect, lasting in some cases for 1-2 minutes after release of the ligature in 1-3-day old puppies. This after-effect shortened with increasing age of the puppies. The magnitude of the hypotensive effect varied according to the age of the animals. It was inconsiderable for 2-3-day-old puppies (a fall of 3-5 mm Hg), and was greater for somewhat older puppies (up to 15 mm Hg). Puppies aged $1^1/_2$-2 months (4) did not display any increase in blood pressure after constricting the vein. No effect on respiration was evident in the great majority of our experiments. Examples of the effects on blood pressure and respiration of constriction of the splenic vein are given by the kymograph tracings of Figures 1 and 2 (1- and 7-day old puppies, respectively).

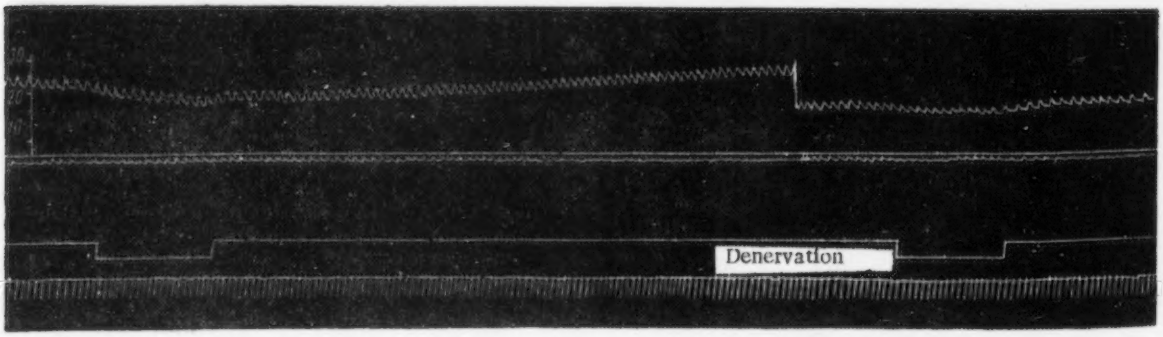


Fig. 1. Changes in blood pressure and respiration following stimulation of splenic receptors by distention of the spleen with blood. Constriction of the splenic vein of a day-old puppy. Explanation of tracings (from above down): blood pressure, base line, respiration, stimulation signals; time marker (3 seconds).

In order to elucidate the reflex nature of the effects observed we repeated the experiments after severing nervous connections with the spleen. All the visible nerve fibers accompanying the splenic artery and vein were cut, and the vessels were painted with procaine or phenol solutions. The effects on blood pressure previously observed were not abolished by denervation, ie., engorgement of the spleen following constriction of the vein, with an intact arterial supply, led to a similar lowering of blood pressure as before denervation.

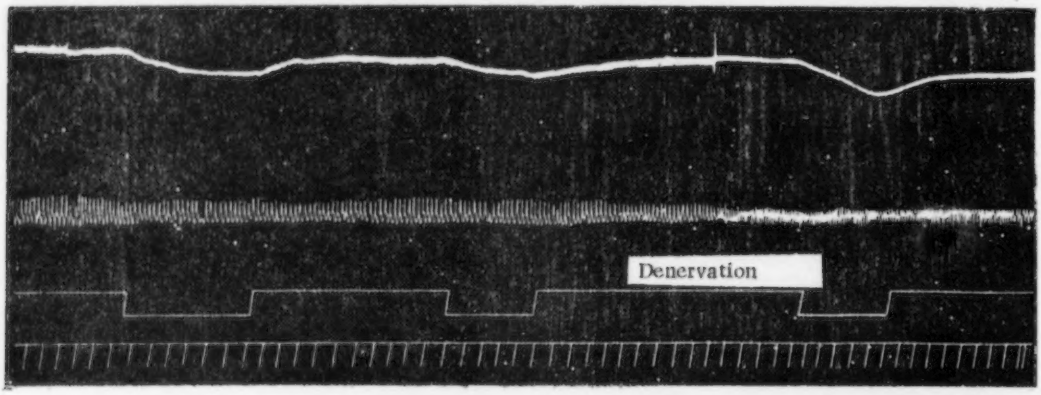


Fig. 2. Changes in blood pressure and respiration following constriction of the splenic vein of a 7-day old puppy. Explanation of tracings (from above down): as in Figure 1. Time marker (5 seconds).

Evidently the fall in arterial pressure observed after constriction of the splenic vein of very young animals is a consequence of the passive reception of blood by the spleen, which acts as a blood depot at this age, and which also releases it passively, as is evidenced by the prolonged after-effect found after release of the ligature from the vein.

In the 2nd series of experiments engorgement of the spleen was achieved by raising the pressure within the splenic vessels.

The spleen, after isolation from the systemic circulation, and hence from humoral factors, was perfused, through its main artery, with oxygenated Ringer-Locke solution at 37-38°. Pressure was raised within the limits 60-110 mm Hg by elevating the reservoir containing perfusion fluid. Higher pressures were achieved by forcing liquid in by means of a syringe. Pressure was measured by means of a mercury manometer.

In the 13 experiments of this series, on puppies aged from 17-20 days to 2 months, stimulation of the baroceptors of the splenic vessels had no effect on arterial pressure and respiration.

With puppies aged from 2 to 4 months, in half of the experiments performed raising the perfusate pressure to 80 to 110 mm Hg caused a slight rise in carotid artery pressure, but had no effect in the other half. More considerable changes in carotid pressure resulted from greater elevations of perfusate pressure, achieved by injection with a syringe.

The absence of reflex reactions from splenic baroceptors of young puppies, acting on blood pressure and respiration, may be deduced from the results of our experiments. The changes in blood pressure observed following stimulation of the baroceptors of the spleen of adult animals were seen in our experiments on 2-3-month old puppies.

These findings are in accordance with those of A. P. Polosukhin [2, 3], who found that reflex regulation of the volume of the spleen is established at the age of $2^1/_2$-3 months, up to which age the spleen takes no active part in the processes of distribution of blood in the organism.

The absence of any reflex regulation through changes in arterial pressure under conditions of splenic engorgement in young puppies may be ascribed to the functional immaturity of the nerve centers, since it is known from the literature that the terminal ramifications of the splenic nerves are capable of conducting and transmitting stimuli at birth.

The third series of experiments was on the effects on blood pressure and respiration of stimulating splenic chemoceptors.

Solutions containing 30 to 100 μg of histamine or acetylcholine were introduced into spenic vessels with intact innervation. The solutions were injected through the walls of the rubber tubing attached to the perfusion cannula. In order to avoid excessive dilution of the reagents the flow of perfusion fulid was discontinued during the injection. We performed 16 experiments in this series.

Owing to the difficult anatomical approach to the main trunks of the spenic artery and vein in very young animals we were obliged to do the perfusion experiments on puppies aged 17-20 days or more.

In the great majority of experiments introduction of histamine caused a rise in blood pressure. In 10 of the 16 experiments there was no effect on respiration. In some of the experiments we observed a diminution in the amplitude of the respiratory movements, without change in frequency, or with slight retardation. Acetylcholine also affected blood pressure, but at higher dosage levels, and the effects were less pronounced than with histamine. Respiration was unaffected.

As is evident from Figure 3, introduction of histamine or acetylcholine into the splenic vessels of a month-old puppy led to a rise in blood pressure.

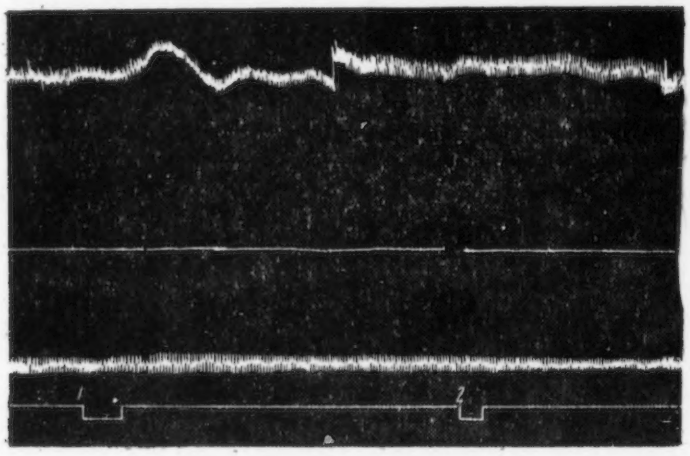


Fig. 3. Changes in blood pressure and respiration following stimulation of splenic receptors by means of introduction of histamine (1) or acetylcholine (2) into a month-old puppy.
Explanation of tracings (from above down): as in Figure 1.

Our experiments thus show that stimulation of the splenic chemoceptors of 17-20 day-old puppies causes changes in blood pressure and repiration. The development of these reflexes at an earlier age is not excluded by our experiments, since we could not perform splenic perfusion of younger puppies.

Our findings show that reflex vasomotor and respiratory reactions to stimulation of splenic baroceptors, characteristic of adult animals, are absent at birth, and first appear at the age of $2^1/_2$-3 months.

Interoceptive effects from splenic chemoceptors are demonstrable at an earlier age.

LITERATURE CITED

[1] M. I. Kokhanina, Izvest. Akad. Nauk Kazakh. SSR, Ser. Fiziol., No. 73 (2), 111-119 (1919).

[2] A. P. Polosukhin, Development of Humoral and Reflex Regulation of the Spleen, as a Blood Depot, in Ontogenesis,* Thesis, 1939.

* In Russian.

[3] Ibid., Byull. Eksptl. Biol. i Med., 6, No. 2, 216-219 (1938).

[4] E. G. Skipina, Byull. Eksptl. Biol. i Med , 39, No. 6, 14-18 (1955 .

[5] E. G. Skipina, Reflex Effects on Venous Pressure from Visceral Reactors.* Thesis, Alma-Ata (1952).

[6] V. N Chernigovksy, Afferent Systems of the Viscera,* Kirov, 1943.

EXPERIMENTAL PITUITRIN HYPERTENSION AND CORONARY INSUFFICIENCY IN MONKEYS

A. A. Belous and G. O. Magakyan

From the Sukhumi Medicobiological Station, Acad. Med. Sci. USSR (Director: I. A. Utkin), and the Department of Pharmacology, Institute of Experimental Medicine (Director: Prof. S. V. Anichkov, Member Acad. Med. Sci. USSR), Acad. Med. Sci. USSR

(Received April 16, 1956. Presented by S. V. Anichkov, Member Acad. Med. Sci. USSR)

It has been shown by A. A. Belous [1, 2] that daily intravenous injection of 0.5 ml of pituitrin for 13-15 days gives rise to persistent hypertension in dogs and rabbits. This author noted that pituitrin causes changes in cardiac activity. A comparison of the electrocardiograms taken before and after pituitrin treatment of dogs showed changes in the activity of the heart typical of coronary insufficiency. In rabbits, the electrocardiograms showed disturbances indicative of myocardial dystrophic change (arrhythmia, bradycardia, change in the electrical axis of the heart, lowered potentials).

Although experimental pituitrin hypertension in dogs and rabbits is not identical with the human condition, there are nevertheless grounds for believing that changes in the functioning of the endocrine glands, in particular the neurohypophysis, are of considerable importance in the pathogenesis of human hypertensive disease.

We thought it of great importance for the elucidation of the role of pituitrin in the causation of hypertensive disease to study the possibility of inducing pituitrin hypertension, and also the effects of pituitrin on the action of the heart of monkeys.

According to the reports of the Sukhumi Medicobiological Station [6, 7], spontaneous hypertension with symptoms of coronary insufficiency, resembling the picture seen in human hypertensive disease, is not infrequently encountered among monkeys maintained under artifical conditions. We attempted to establish whether pituitrin-induced hypertension with cardiac disturbances could be produced in monkeys, in the same way as in rabbits and dogs.

EXPERIMENTAL METHODS

The experimental animals consisted of 9 rhesus monkeys aged from $3\frac{1}{2}$ to 12 years, including 3 females and 6 males. Irrespective of age and weight all the monkeys were given one intravenous injection daily of 0.5-0.6 ml of pituitrin, for 18-22 days, except in one case (the monkey Karlik) in which only 12 injections were given when persistent hypertension set in. Blood pressure was measured daily in vessels of the forelimbs by means of a Riva-Rocci instrument, for 3 days before the course of injections, during the injection period, and after the injections had been discontinued. Electrocardiograms were taken over the whole of the observation period.

EXPERIMENTAL RESULTS

The initial blood pressure varied from 130/70 to 150/100 mm for 6 monkeys (Forum, Artam, Nil, Perepel, Vilya, Vanessa), and from 160/100 to 170/100 mm for 3 monkeys (Zlatka, Slepen', Karlik). Rise in blood pressure was observed after 4-8 injections, but it was not persistent. For this reason injections were continued

*In Russian.

until the blood pressure reached a constant high level. Daily measurement of blood pressure after discontinuance of pituitrin injections showed that persistent hypertension had developed in 7 monkeys (4 normotensives and 3 initially hypertensives), persisting over a 7-day period after the last injection. Hypertension did not develop in 2 monkeys (Vanessa and Perepel); Perepel exhibited elevated blood pressure during the injection period, with a return to the initial level immediately after they were discontinued. Subesequent successive measurments (2 and 7 months later) showed that blood pressure had returned to normal in 2 of the 7 hypertensive monkeys, while hypertension still persisted after a further 7 months in 5 monkeys (3 initially normotensive, 2 initially hypertensive). The object of the experiments on the initially hypertensive animals was to ascertain whether administration of pituitrin caused any further rise in pressure, and, what would be of even greater interest, whether it would affect cardiac activity. The variations in blood pressure of monkeys receiving pituitrin injections are presented in Figure 1.

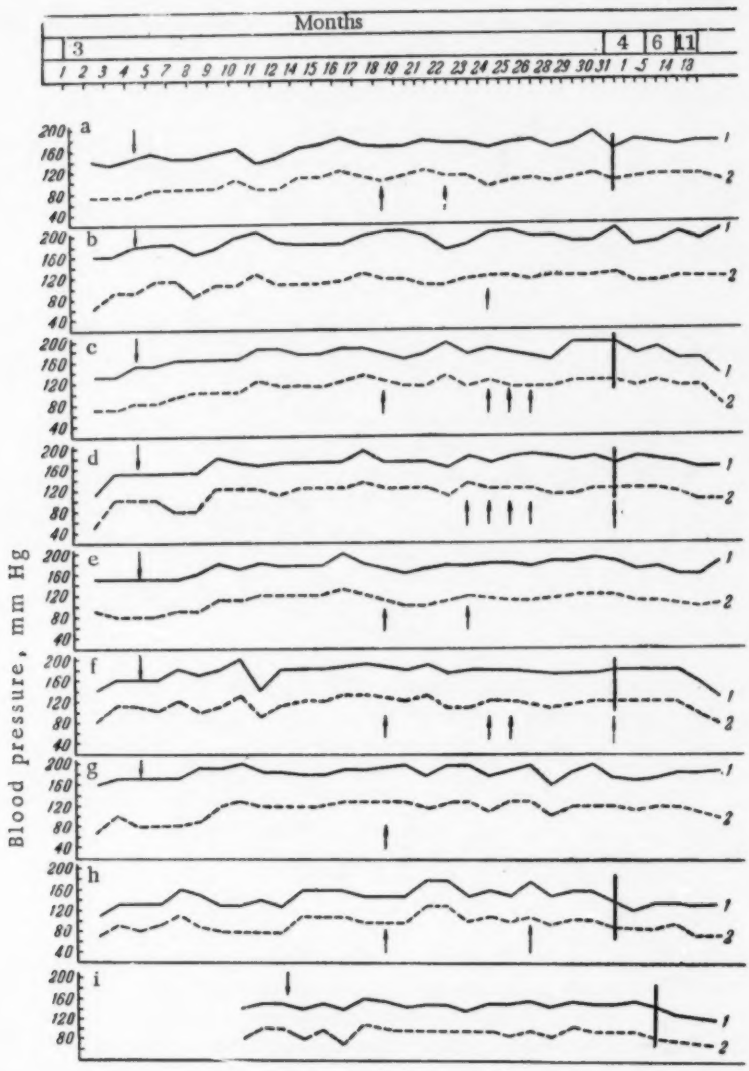


Fig 1. Variations in arterial pressure of monkeys receiving intravenous injections of pituitrin (March-November 1955). 1) aximum blood pressure; 2) minimum blood pressure; ↓ pituitrin injections commenced; vertical line – pituitrin injections discontinued; ↑ injection not given. Blood pressure curves for the monkeys: a) Forum 12 years old; b) Slepen 8 years; c) Nil 10 years; d) Vilya 5 years; e) Artam 5 years; f) Zlatka 7½ years; g) Karlik 7 years; h) Perepel 6 months; Vanessa 7½ years old.

The data of Figure 1 show that persistent hypertension developed in 7 of 9 injected monkeys.

Electrocardiographic study of the effects of pituitrin on the action of the heart showed the following: the preinjection of electrocardiograms showed no deviation from normal in 6 animals, and slight dystrophic myocardial changes in the other 3.

After daily pituitrin injections (for 15-26 days) electrocardiographic changes were seen, starting with the 11th-16th day in 6 animals, of the coronary insufficiency type in Nil, Forum, Karlik and Slepen (inversion of the T-wave with the standard and IV pectoral leads—(Fig. 2). Slepen displayed ventricular extrasystoles (Fig. 3).

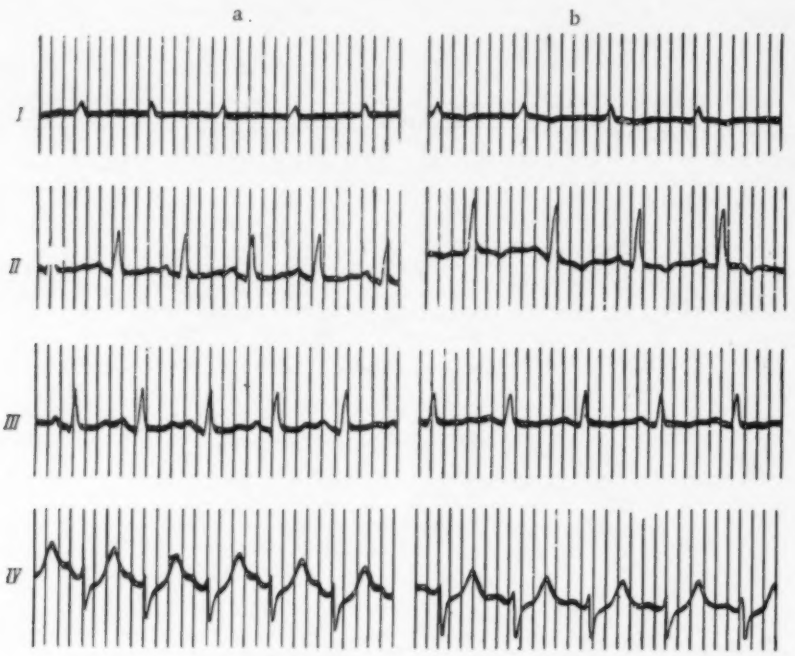


Fig. 2 Electrocardiograms for the monkey Forum a) Before, and b) after, introduction of pituitrin; Leads I, II, III, and IV.

Disturbances of cardiac activity appeared in one monkey (Vilya) on the 15th day; the E.C.G. shows auricular extrasystoles with lead III, and these were also seen 2 weeks after discontinuing the injections, with the IV pectoral lead. The monkey Perepel did not develop persistent hypertension, but only transient increase in pressure during the injection period; the electrocardiogram was indicative of dystrophic myocardial changes, viz., appearance of inverted T- and P-waves with standard lead III. The changes in the activity of the heart noted above persisted for a long time after pituitrin had been discontinued (over 2 to 7 months of observation). The ECG showed no deviations from the normal in 3 monkeys (Zlatka, Artam, and Vanessa).

DISCUSSION OF RESULTS

Our experiments show that it is possible, by means of intravenous injections of pituitrin, to induce hypertension in monkeys, as has been shown previously for dogs and rabbits [1, 2]. Our findings support the view advanced by numerous workers [3, 4, 5, 8, 9], that the endocrine glands may play a part in the pathogenesis of hypertensive disease, and in particular the hormones of the posterior lobe of the hypophysis. Our electrocardiographic studies showed that the changes in cardiac activity occurring after prolonged administration of pituitrin are of the type encountered in coronary insufficiency, and make their appearance about 5-7 days after the inception of hypertension.

It is not clear whether the cardiac disorders are ascribable to the direct action of pituitrin on the blood vessels and conducting system of the heart (inversion of the T-wave, and extrasystoles), or whether they are a

secondary consequence of the hypertension. Hypertension did not result in the monkey Perepel, which showed dystrophic myocardial changes (inverted T- and P-waves, in lead III). This is evidence that pituitrin can, independently of causing hypertension, exert a direct action on the coronaries, giving rise in the first place to dystrophic changes, from which result changes in cardiac activity of the type of coronary insufficiency.

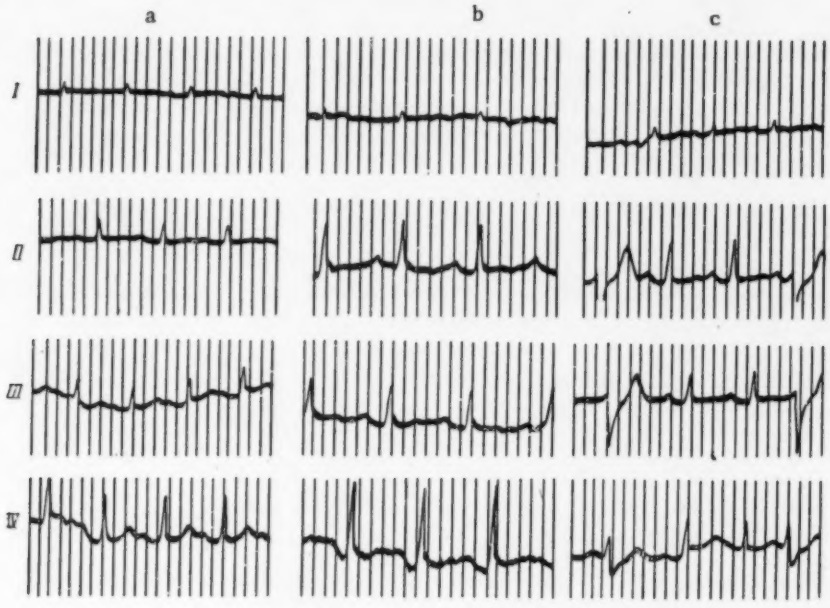


Fig. 3 Electrocardiograms for the monkey Slepen . a) Before injecting pituitrin, b) on the 14th day of injection, c) on the 5th day after discontinuing injections; Leads I, II, III, and IV.

Taking into account published evidence that hypertensive disease is frequently associated with coronary insufficiency, it may be concluded from our results that posterior pituitary hormones may, among other factors, be responsible for the origination of hypertension and cardiac disorders.

LITERATURE CITED

[1] A . A. Belous and N. A Erofeeva, Pharmacology of New Therapeutic Agents*, pp. 130-133, Leningrad, 1953.

[2] A. A. Belous, Farmakol. i Toksikol., 17, No. 2, 10-13 (1954).

[3] V. M. Kogan-Yasnyi, V. A. Vartapetov and R. Ya. Spivak, Klin. Med., 15, No. 12, 1382- 1395 (1937).

[4] V. M. Kogan-Yasnyi, Arkhiv. Biol. Nauk SSSR, 51, No. 1-2, 5 (1938).

[5] Ibid., Byull. Eksptl. Biol. i Med., 6, No. 3, 363-367 (1938).

[6] G. Ya. Kokaya, Byull. Eksptl. Biol. i Med., 38, No. 12, 23-27 (1954).

[7] G. O. Magakyan, Byull. Eksptl. Biol. i Med., 35, No. 2, 44-45 (1953).

[8] A. L Myasnikov, Classification of Hypertensive Disease,* Moscow, 1951.

[9] E. M. Tareev, Hypertensive Disease,* Moscow, 1948.

* In Russian.

REGULATION OF DISORDERED RHYTHM OF THE HEART BY MEANS OF PROCAINE BLOCK OF EXTRACARDIAL NERVOUS FORMATIONS, AND OF OTHER AGENTS AFFECTING THE NERVOUS SYSTEM

B. M. Fedorov

From the Institute of Normal and Path. Physiology (Director: V. N. Chernigovskii, Member Acad. Med. Sci. USSR), Acad. Med. Sci. USSR, Department of General and Experimental Pathology (Director: Academician A. D. Speransky), Oscillographic Division (Director: E. A. Gromova), and Laboratory for Experimental Therapy (Director: A. M. Chernukh)

(Received February 8, 1956. Presented by Academician A. D. Speransky)

Numerous workers, both clinicians and physiologists, have remarked on the dependence of qualitative alterations in heart rhythm in disorders of the heart on nervous stimuli. The experiments of E. E. Epshtein [9], from V. N. Chernigovsky's laboratory, are of special interest; these experiments showed that very profound alterations in cardiac rhythm may result from stimulation of alimentary tract interoceptors, when the heart is under the action of calcium chloride.

The present paper deals with an investigation of the possibility of restoring normal rhythm in a number of types of cardiac arrhythmia, by means of factors affecting the nervous system. It should be noted that in different disorders of heart rhythm the effect of one and the same factor depends very largely on the nature of the arrhythmia, and on the actual mechanism of the process leading to its development.

We have shown in an earlier paper [7] that in strophanthin poisoning the same reflex reaction which favorably affects atrioventricular conductivity and raises myocardial excitability does not act in the same way in different types of arrhythmia. This reflex reaction totally abolishes all disturbances of atrioventricular conductivity in atrioventricular block of the second degree, but it aggravates arrhythmia in some cases of heterotopic rhythm.

The dependence of the effect of interference with nervous mechanisms on the nature of the given cardiac arrhythmia was very clearly shown in our experiments involving interference with extracardial nervous formations.

EXPERIMENTAL METHODS AND RESULTS

Cardiac arrhythmia was produced in experimental animals by administration of toxic doses of strophanthin, and in some cases also by ligature of large blood vessels of the heart (usually the anterior descending coronary artery).

The short experiments were most often performed under morphine-ether-chloroform anesthesia, and some isolated experiments under Barbamyl anesthesia. The dogs were given different intravenous doses of Strophanthin K. In order to bring about the gravest form of arrhythmia–paroxysmal ventricular tachycardia– we injected doses of 0.145 mg strophanthin per kg body weight. This gave rise to the expected effect.

It appeared from our experiments on strophanthin-poisoned dogs subjected to various forms of interference with the vagus nerves that the effect observed varies according to the acuteness of poisoning and with the given stage of development of arrhythmia. With comparatively light degrees of intoxication, with retention of the sinus rhythm of the heart, we observed that stimulation of the vagus nerves caused retardation of the sinus rhythm, impairment of atrioventricular conductivity, amounting in some cases to total block, and appearance of ventricular extrasystoles; ventricular rhythm, which is a feature of strophanthin poisoning, is also observed.

Severing the vagus nerves, or injecting procaine into them, led to a normalization of the heart rhythm, both in atrioventricular block and with heterotopic ventricular rhythm of comparatively low frequency. Exclusion of vagus nerve influences always caused restoration of atrioventricular conductivity in dogs with incomplete atrioventricular block, due to moderate degrees of strophanthin poisoning. We also found that abolition of vagus

effects restored normal heart rhythm in cases of heterotopic ventricular rhythm of 60-120 beats per minute.

Restoration of sinus rhythm was only exceptionally encountered following vagotomy in cases of severe strophanthin poisoning, in which the ventricular rhythm did not, however, exceed 200 beats per minute. With ventricular rates exceeding 200, interruption of vagus connections in no case restored sinus rhythm. Very large doses of strophanthin were given to a group of 20 dogs, 10 of which had previously been subjected to vagotomy, and the remaining 10 were vagotomized when the ensuing arrhythmia was at its height. In no case did preliminary vagotomy prevent the development of severe disturbances of cardiac rhythm: 9 dogs developed extrasystolic ventricular tachycardia (Figure 1), and one dog showed extrasystolic coupling of heart beats.

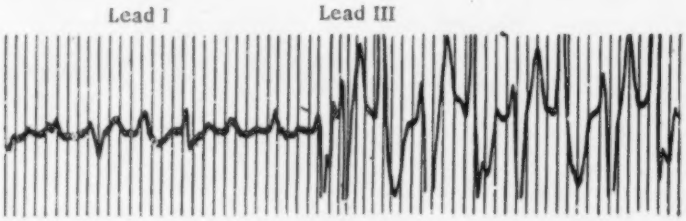


Fig. 1. Polytopic extrasystolic ventricular heart rhythm after administration of strophanthin to previously bilaterally vagotomized dogs; leads I and III.

When vagotomy was performed at the height of development of paroxysmal ventricular tachycardia, with a rate of 190-240 beats per minute, we observed a return to sinus rhythm in only one dog, with a ventricular rhythm of about 200 beats per minute. Restoration of normal rhythm was not observed in the other 9 cases.

Vagus stimulation in the most severe cases of strophanthin poisoning also had no effect on the heart rate. This finding is in full accord with I. P. Pavlov's experimental results; he found that vagal stimulation did not lower the frequency of the heart beat in dogs suffering from acute poisoning with the cardiac glucoside of Convallaria majalis.

It thus appears that in fully developed paroxysmal ventricular tachycardia restoration of normal heart rhythm cannot be achieved either by stimulation or by section of the vagus nerves. Even the most accelerated sinus rhythm possible after vagotomy does not in these cases exceed the ventricular heterotopic beat rate, and the disturbances in conductivity may depend not only on changes in the conducting system of the heart, and on heightened tonus of the vagus nerves, but also on the presence of simultaneously functioning foci of excitation in the auricles and ventricles (we have never, in the course of development of arrhythmia, seen deterioration of atrioventricular conductivity due to ventricular extrasystoles).

It is common clinical practice to apply stimulation of the vagus nerves to cut short attacks of auricular paroxysmal tachycardia [1, 3, 4, 8]. It is generally believed that suppression of the extrasystolic rhythm of ventricular paroxysmal tachycardia by means of vagus stimulation is not possible. We were, however, able to discern a stage in the development of arrhythmia in which stimulation of the vagus nerves causes definite inhibition of ventricular foci of excitation.

Only for an hour after administration of highly toxic doses of strophanthin, when the first signs of depression of the activity of ventricular automatism appear, does vagus stimulation cause change in the heart rhythm, by exerting an inhibitory effect on the foci of heterotopic excitation in the ventricles (more pronounced than in the sinus node).

In particular, we found that stimulation of the peripheral segment of a severed right vagus nerve, using an induction current, caused inhibition of both the heterotopic focus of excitation of ventricular rhythm and the sinus node. A very transient restoration of sinus rhythm was observed after terminating stimulation of the nerve. Stimulation of the left vagus nerve caused suppression of the focus of excitation of heterotopic ventricular rhythm, with retention of auricular sinus rhythm, and full atrioventricular block (Figure 2).

Stimulation of the same nerves at the stage of fully developed arrhythmia was without effect.

We were, however, able to restore normal sinus rhythm at the height of the most acute paroxysm of ventricular tachycardia, by acting on elements of the nervous system. Attempts have been made to treat paroxysmal tachycardia by surgical intervention, applied to the sympathetic nervous system [11].

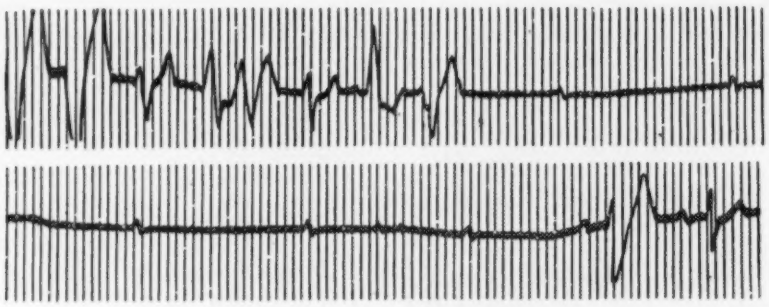


Fig. 2. Suppression of heterotopic ventricular rhythm, with retention of sinoauricular rhythm and full atrioventricular block, during stimulation of the peripheral segment of the severed left vagus nerve.

Of the drugs applied to the treatment of arrhythmias connected with enhanced automatism of the heart, wide use has been made of quinidine, and, more recently (according to the foreign literature), of procaine amide. Some authors [13] relate the effect of intravenous procaine amide to its direct action on the heart, while others believe that it has a sympatholytic action [12]. Procaine amide is not, however, efficaceous in all cases [10].

In view of the successful introduction of procaine block, which has, thanks to the work of A. D. Speransky [5, 6], been widely applied to the treatment of a number of different diseases, and on the basis of the concepts developed by this author, we undertook the study of the effects of procaine block of extracardial nervous formations on cardiac arrhythmias caused by administration of highly toxic doses of strophanthin, or by ligature of major coronary arteries. We performed 4 groups of experiments, in which strophanthin was administered at dosage levels of 0.145 mg per kg body weight.

In the first, control group of experiments we gave strophanthin alone to a group of 6 dogs, without applying any other measures. All the animals developed ventricular paroxysmal tachycardia, the rate of the ventricular rhythm attaining 200-240 beats per minute 20 minutes after the injections.

The second group of 6 dogs received strophanthin at the same dosage levels, after preliminary procaine block of the vagosympathetic trunks and the stellate and upper thoracic sympathetic ganglions. Normal sinus rhythm was found in all cases after administration of strophanthin.

The third group of 12 dogs was given strophanthin at the same dosage levels as before, and paroxysmal ventricular tachycardia was allowed to develop, after which procaine block of extracardial nervous formations was instituted. Sinus rhythm was restored in all cases 5-10 minutes after the block had been established (Figure 3). The sinus rhythm was normal in 10 cases, and in the other two the paroxysms of ventricular tachycardia underwent conversion to sinus rhythm, but disturbances of atrioventricular and intraventricular conductivity persisted for a certain time.

In the fourth group of 6 dogs, intravenous injection of strophanthin was combined with synchronous intramuscular injection of procaine. We observed that intramuscular administration of procaine depresses the activity of heterotopic ventricular foci, reducing the ventricular rhythm from 200-220 to 100-130 beats per minute. Restoration of normal rhythm was not, however, observed in any of the animals of this group.

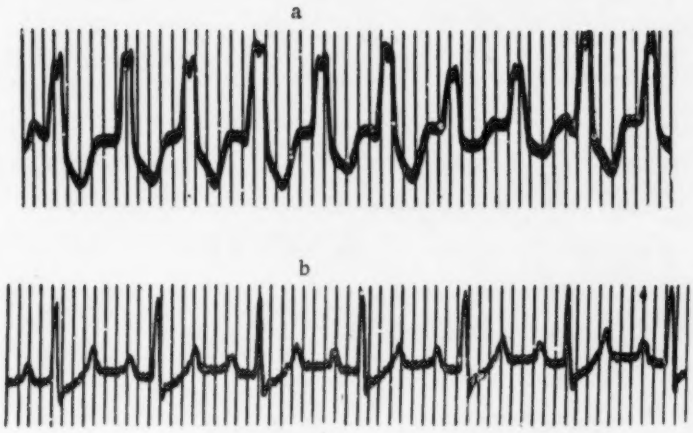


Fig. 3. Restoration of normal heart rhythm following procaine block of extracardial nervous formations.
a) Right ventricular extrasystolic rhythm of the heart following administration of strophanthin; b) sinus rhythm following procaine block of the vagosympathetic trunks and upper thoracic sympathetic ganglions.

Bearing in mind the complexity of the physiological changes taking place in the organism following introduction of considerable amounts of procaine in the vicinity of the vagosympathetic trunks and the upper thoracic sympathetic ganglions, we attempted to act on the sympathetic innervation of the heart in a more selective way, by introducing 2 ml of 2% procaine solution subdurally into the cerebrospinal fluid, at the level of the 2nd thoracic segment. Subsequent injection of highly toxic doses of strophanthin did not give rise to ventricular tachycardia, although conductivity disturbances were observed.

It thus appears that procaine block of the extracardial sympathetic formations and vagus nerves prevents the development of premature beats and of ventricular tachycardia, as well as of disturbances of conductivity. Subdural injection of procaine prevents paroxysmal ventricular tachycardia, but does not avert conductivity disturbances.

We also examined the effect of procaine block of the vagosympathetic trunks and the upper thoracic sympathetic ganglions on extrasystolic ventricular arrhythmia resulting from high ligature of the anterior descending coronary artery. It was found that procaine block of extracardial nervous formations restored normal rhythm (at least for a number of hours) in dogs suffering from impaired coronary circulation, with an extrasystolic heart rhythm of high frequency. This effect was also achieved at those stages of development of extrasystolic ventricular arrhythmia not responding to interruption of vagus nerve impulses alone.

Certain possible side-effects may result from the application of procaine block of extracardial nervous formations. These may appear during the first few minutes after injection of procaine. Rapid injection of procaine into the vicinity of both vagosympathetic trunks and of the stellate ganglions may cause arrest of respiration. Forced respiration should then be instituted, and can save the life of the animals if it is begun in good time, and maintained for several minutes.

A second complication is the possibility of rapid suppression of ventricular automatism before atrioventricular conductivity has been re-established.In one of our experiments we found that procaine block was immediately succeeded by an asystolic state of the ventricles, lasting for several seconds, until partial restoration of atrioventricular conductivity supervened.

It may be concluded from the results of our experiments that procaine block of extracardial nervous formations is very effective in the treatment of severe cardiac arrhythmias. It is probable that blocking sympathetic formations depresses ventricular automatism, while elimination of vagus nerve influences enhances atrioventricular conductivity.

LITERATURE CITED

[1] V. F. Zeleny, Diseases of the Heart Characterized by Disturbance of Rhythm,* Moscow, 1915.

[2] I. P. Pavlov, Centripetal Nerves of the Heart,* St. Petersburg, 1883.

[3] A. M. Sigal, Heart Rhythms and their Disturbances,* Odessa, 1935.

[4] L. Ya. Siterman, Disturbances of Heart Rhythm,* Minsk, 1935.

[5] A. D. Speransky, Elements for the Construction of a Theory of Medicine,* VIEM Press, 1937.

[6] Ibid. Nervous Trophism in the Theory and Practice of Medicine,* Moscow, 1936.

[7] B. M. Fedorov, Byull. Eksptl. Biol. i Med., 37, No. 2, 17-22 (1954).

[8] L. I. Fogelson, Diseases of the Heart and Blood Vessels,* Moscow, 1951.

[9] E. E. Epshtein, Trudy Voenno-morsk. med. akad., 2, 63-74 (1951).

[10] R. Charlier, Acta clin. belg., 1953, Vol. 8, No. 1, pp. 9-32.

[11] D. Danielopolu, Arch. d. maladies du coeur, des vaisseaux et du sang, 1932, ann. 25, pp. 577-604.

[12] H. Miller, M. Nathanson and S. Griffith, J. Am. Med. Assoc., 1951, Vol. 146, No. 11, pp. 1004-1007.

[13] D. Schert, Circulation, 1953, Vol. 8, No. 5, pp. 756-768.

FUNCTIONAL DISTURBANCES FOLLOWING MASSIVE INFUSIONS OF PHYSIOLOGICAL SALINE

G. L Lyuban

From the Chair of Pathological Physiology (Director: Prof. G. L. Frenkel), Kirgiz State Medical Institute (Director: Assistant Professor A. A. Aidaraliev)

(Received January 19, 1955. Presented by V. N. Chernigovsky, Member Acad. Med. Sci. USSR)

The sensitivity of the organism to loss of blood is not dependent solely on the amount of blood lost, or on the rate at which it is lost. Experiments carried out in I. R. Petrov's laboratory [3] have shown that over-heating, trauma, starvation, and other factors greatly aggravate the consequences of hemorrhage. Loss of blood leads more quickly to hemorrhagic collapse in anesthetized [1] or shocked [2] subjects. Replacement therapy, involving transfusion of blood or blood extenders, is most effective during the early stages of collapse. Infusions of blood or physiological saline are given after loss of blood in order to avert collapse. Saline is sometimes infused in very large amounts, with the intention of providing the organism with the largest possible reserves of fluid.

Arterial pressure is not significantly affected by slow intravenous infusion of even very large amounts of physiological saline. On this basis it has been thought that massive infusions of physiological saline are quite safe, and are therefore not only permissible, but even desirable in cases in which there is a risk of hemorrhage. It should not, however, be overlooked that intravenous infusion of physiological saline is not without effect on the reaction of the organism to loss of blood.

We have investigated the reaction of the organism to loss of blood following intravenous infusion of large volumes of physiological saline.

* In Russian.

EXPERIMENTAL METHODS*

Our experiments were performed on dogs. Subcutaneous injections of morphine, in doses of 0.5 ml of 1% solution per kg of body weight, were given 30-45 minutes before the experiment. The dogs were tied down, and the common carotid artery, trachea, external jugular vein, and iliac artery were exposed. Recordings of arterial pressure and respiration were made during the experiments. A glass cannula was inserted into the jugular vein, and connected by rubber tubing with a graduated vessel containing physiological saline at 37-39°. After recording the initial arterial pressure and the respiration, the physiological saline was infused into the vein, at a rate of 10-15 ml per minute, until the total volume infused amounted to 1-2 times the volume of circulating blood (this volume was computed from I. R. Petrov's table). Immediately after completion of the transfusion blood was withdrawn through a cannula inserted into the iliac artery.

EXPERIMENTAL RESULTS

Since slow infusion of large amounts of saline had no significant effect on arterial blood pressure, blood letting began at the pressure level shown in Table 1. The animals were in a state of profound hemorrhagic collapse. Arterial pressure fell to 10-40 mm Hg after the loss of 29-69% of the circulating blood.

The results are presented in the Table.

TABLE 1

Effect of Saline Infusions on the Sensitivity of the Organism to Subsequent Exsanguination

Weight of dog, Kg	Initial arterial pressure, mm Hg	Amount of physiological saline infused, ml	Arterial pressure after infusion, mm Hg	Amount of blood removed, ml	Percentage of total volume of blood removed	Arterial pressure after exsanguination, mm Hg
7.3	124	1 100	104	184	35.3	30
7.1	150	570	150	150	29.4	24
6.2	150	600	150	230	51.9	40
7.4	140	700	150	300	50.7	25
7.0	120	600	126	300	60.0	10
8.0	160	1 100	164	375	65.6	30
8.9	150	900	160	440	69.1	25
5.3	160	700	150	200	65.1	16
11.7	175	900	200	700	66.6	24
20	150	2 000	160	1 000	64.6	30
			Average		56.6	

As is evident from the Table, massive infusion of physiological saline not only did not raise resistance to exsanguination, but in fact lowered it. Profound shock followed the loss of about half the circulating volume of the blood.

It appears that the nature of the reaction of the arterial pressure to loss of blood changes after saline infusions. Arterial pressure remained steady for a certain time after blood-letting had begun, in the control animals (Figure 1). Fall in arterial blood pressure began as soon as bleeding of previously infused animals was commenced. This is evidence of weakening of compensatory reflex reactions, which usually appear in response to loss of blood. The alterations in arterial pressure recorded during exsanguination of an animal previously loaded with saline are shown in Figure 2.

It thus appears that although slow, massive infusion of physiological saline does not give rise to any visible stable changes in arterial pressure it nevertheless affects the reactivity of the organism.

* The Third-Year students Novikova and Ryzhkova took part in these experiments.

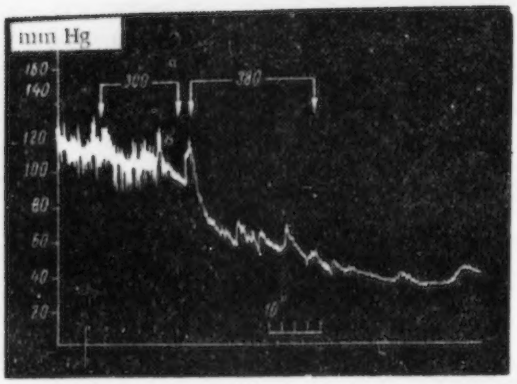


Fig. 1. Alterations arterial pressure of a healthy dog during exsanguination (weight of dog 11 kg).

The absence of pressure changes during slow infusion is evidence of the rapid deployment of reflex adaptive mechanisms, tending towards maintenance of the normal level of arterial pressure. The more prolonged the infusion, the greater is, in all probability, the increase in sensitivity of the vasomotor center, and the more active and adequate is the corresponding compensatory reaction.

It might have been expected that at higher rates of infusion of saline the compensatory reaction would also be accelerated, and hence that there would be a steadily rising intensification of stimulation of the vasomotor center. The possibility thus emerges of development of inhibition of the center, presumably of a parabiotic nature, with the danger of fall in arterial pressure.

The following experiments* were performed on a group 12 dogs, in order to verify this possibility. The animals were treated as in the preceding group, except that intravenous infusion of saline took place at a rate of not less than 50 ml per minute; as before, pressure was registered in the common carotid artery.

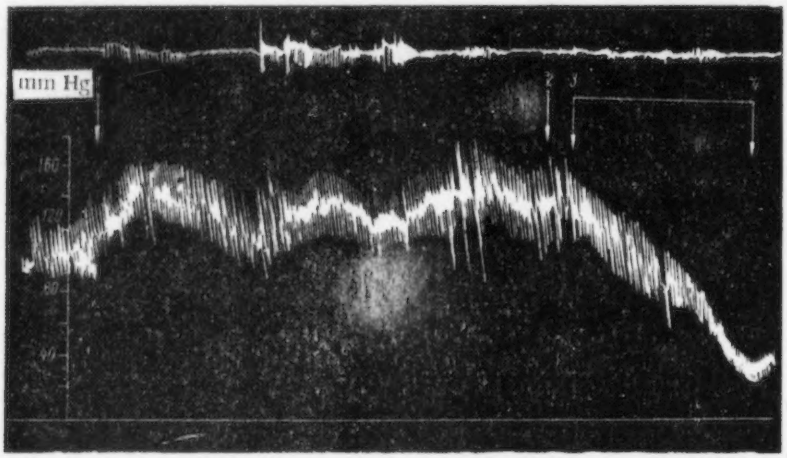


Fig. 2. Reaction to exsanguination of a dog previously infused with physiological saline (weight of dog 6.2 kg). 1) Beginning of infusion; 2) end of infusion; 3) beginning of blood-letting; 4) end of blood-letting. 500 ml of physiological saline was infused, and 230 ml of blood removed. Explanation of curves (from above down): respiration, arterial pressure.

The reaction elicited in response to acute overloading with saline, in particular that part of it manifested by alterations in arterial pressure, was not uniform for all the animals. In two cases arterial pressure at first rose steeply, and then fell abruptly, leading to the death of the animal (Figure 3).

A considerable fall in arterial pressure was seen in 7 dogs soon after beginning the infusion. The dogs did not die, however, and the pressure even rose slightly as infusion was continued. Death ensued as a result of pulmonary edema when the volume of fluid infused was equal to 2-3 times the volume of circulating blood. Pulmonary edema supervened in 3 dogs without previous fall in arterial pressure.

* The Third Year students Chaika, Rudneva, and Lizogubenko took part in these experiments.

It thus appears that rapid infusion of physiological saline caused a pronounced fall in arterial pressure in 9 out of 12 dogs, followed by their death, i.e., collapse ensued from acute overloading of the organism with physiological saline.

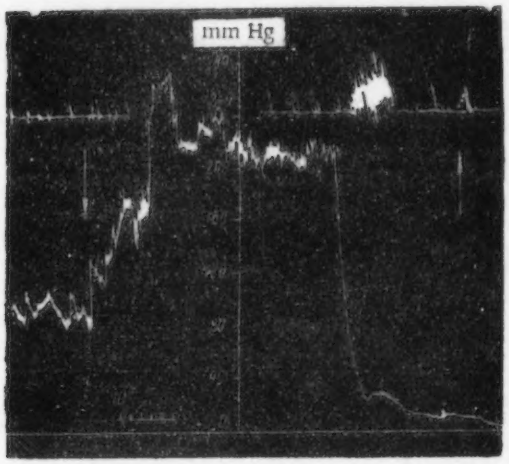


Fig. 3. Reaction to rapid infusion of physiological saline (650 ml infused; weight of dog 6 kg). ┤beginning of infusion; ├ end of infusion. Explanation of curves as in Figure 2.

The most common cause of death from acute overloading with physiological saline was pulmonary edema. Arrest of the heart took place at relatively high arterial pressures (80 mm Hg). After opening the iliac artery pressure fell gradually and smoothly to zero. This is evidence of the disturbance in normal hemodynamics caused by overloading with physiological saline.

Histological examination of the organs of the animals of this group, performed in collaboration with the Chair of Pathological Anatomy, revealed certain characteristic changes in the tissues' structure, apart from the generalized edematous condition. The argyrophilic substance of the lung tissues was disintegrated, and the pulmonary blood vessels contained protein floccules, suggesting that the plasma proteins had undergone profound alterations.

Our experiments show that significant changes take place in the organism following rapid infusion of large amounts of physiological saline.

Stimulation of the vasomotor center associated with slow infusion of physiological saline aggravates the outcome of subsequent blood-letting.

LITERATURE CITED

[1] T. E. Kudritskaya, Problems of Reactivity and Shock,* Medgiz, 1952.

[2] G. L. Lyuban, Sov. Zdravookhranenie Kirgizy, No. 1, (1954).

[3] I. P. Petrov, Role of the Nervous System in Oxygen Hunger,* Medgiz, 1952.

RATE OF ELIMINATION OF CONGO RED FROM THE BLOOD STREAM OF DECEREBRATE DOGS

S. A. Chesnokova

From the Chair of Physiology, II Moscow Medical Institute (Director: Prof. E. A. Asratyan, Corresponding Member Acad. Sci. USSR)

(Received October 15, 1955. Presented by S. A. Sarkisov, Member Acad. Med. Sci. USSR)

Ablation of the cerebral hemispheres of dogs causes alterations in the vegetative and somatic functions of the organism, such as the nature of gastric secretion, the activity of the salivary glands, etc. There are also changes in the response of the organism to certain factors: they become more responsive to introduction of hormones [1], and they give a more intense leukocytic reaction to parenterally introduced proteins [2].

There can be no doubt that the reticuloendothelial system (RES) takes part in these reactions.

The present paper is devoted to a study of RES function in dogs before and after decerebration.

* In Russian.

Among others, the Congo red test is applied for assessing the functional state of the RES. According to the majority of workers, the Congo red test gives a measure of the absorptive capacity of the reticuloendothelial cells.

EXPERIMENTAL METHODS

We adopted the techniques of M. P. Nikolaev, modifying the method in accordance with our conditions.

The dogs were given intravenous injections of 2% Congo red solution, at a dosage level of 0.25 mg per kg body weight. The first blood sample was taken 4 minute later from a vein of the contralateral limb, and a second sample was taken after 30 minutes. The blood was centrifuged, and the serum separated. The first sample was more intensely colored than the second. The first sample was diluted with water until its color matched that of the second. The ratio of the amount of water added to the amount of serum in the first test is taken as the Congo red index.

The greater the rate of elimination of Congo red from the blood stream (by absorption into cells of the RES), the more does the first sample have to be diluted to match the second.

The magnitude of the Congo red index is therefore in direct proportion to the rate of elimination of the dye from the blood stream.

We determined the Congo red index in 10 normal dogs and in 7 dogs after ablation of the cerebral hemispheres.

More than 60 experiments were performed.

EXPERIMENTAL RESULTS

We found considerable differences in the rate of elimination of the dye between the two groups of animals. There was very little difference between the colorations of the two samples taken from decerebrate dogs. Unoperated dogs gave much greater differences.

Figure 1 presents the values for the Congo red index for 5 normal and 5 operated dogs. In the former group the index varies from 0.4 to 0.8 (mean 0.6,)whereas it is rarely greater than 0.3 in the latter group, the mean value being 0.2.

We modified the method, as follows, in order to get a fuller understanding of the differences in rate of elimination of Congo red in the two groups.

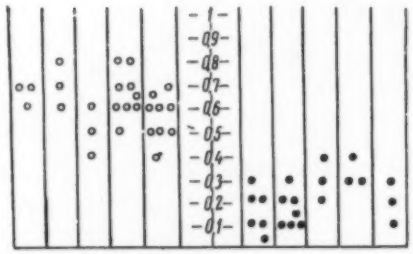


Fig. 1. Value of the Congo red index for unoperated dogs (ieft) and decerebrate dogs (right).

For each experiment we prepared a standard solution of Congo red in serum obtained from the given animal before injection of the dye. The coloration of the standard solution was identical with that of the first sample of serum taken 4 minutes after intravenous injection of the dye. The concentration of Congo red in the standard solution was known, and was taken as the initial concentration in the blood (100%).

Blood samples were then taken at half-hourly intervals during the next 3-4 hours, and the dye content of the serums was determined colorimetrically against the initial value.

The results of the experiments are shown in Figure 2, in which the values for operated and normal dogs are contrasted.

As is evident from the results obtained, elimination of the dye from the blood stream of operated dogs proceeded much more slowly than from normal ones, which is evidence of the lowered activity of the RES cells. Thus, for example, the dye content of the blood of a normal dog had fallen to 28% of the initial value $1^1/_2$ hours after its introduction, as compared with only 66% in a decerebrate dog.

Our experiments show that ablation of the cerebral hemispheres of dogs leads to a fall in the value of the Congo red index, and to a lowering of the rate of elimination of the dye from the blood stream.

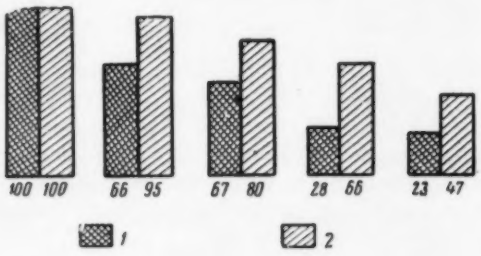


Fig. 2. Alterations in the Congo red content of the blood of normal and decerebrate dogs over 30 minutes after injection of the dye. The concentration of the dye in blood taken 4 minutes after injection was taken as 100%. 1) Unoperated dogs; 2) operated dogs.

This finding points to the existence of a direct or indirect influence of the cerebral cortex on the activity of the RES.

LITERATURE CITED

[1] I. K. Zhmakin, Effect of Ablation of the Cerebral Hemispheres of Dogs on their Gaseous Metabolism,* Thesis, Moscow, 1954.

[2] S. A. Chesnokova, Byull. Eksptl. Biol. i Med., 39, No. 5, 17-19 (1955).

REFLEX CHANGES IN THE INTRACARDIAL NERVOUS FORMATIONS ENCOUNTERED IN FOCAL EXPERIMENTAL MYOCARDITIS

V. S. Livshits and L. N. Lebedeva

From the Comparative Pathology Laboratory (Director: S. I. Franshtein), Institute of General and Experimental Pathology (Director: Academician A. D. Speransky), and the Pathological Morphology Laboratory (Director: Prof. A. A. Solovev, Corresponding Member Acad. Med. Sci. USSR), Institute of Normal and Pathological Physiology (Director: V. N. Chernigovsky, Member Acad. Med. Sci. USSR).

(Received September 12, 1955. Presented by V. N. Chernigovsky, Member Acad. Med. Sci. USSR)

As we have shown in an earlier communication [4], focal myocarditis, produced in dogs and rabbits by application of heat to the apical myocardium, is associated with alteration in the response of the heart to electrical stimulation of the vagus nerves, whereas the response to stimulation of the sympathetics is unaffected.

* In Russian.

It was concluded, from an examination of the experimental material, that the effect was due to impulses proceeding from the pathological focus in the myocardium, leading to a parabiotic state, which, in the first place, affected the least labile nodes of the endocardial nerve formations, resulting in alterations in transmittance of impulses from the vagus to the heart.

Our experimental findings, and their interpretation, have been confirmed by other workers [1, 5, 6].

The experiments of these authors did not, however, exclude the possibility of the direct action of toxic substances on the intracardial nervous formations, and, indeed, assumed that such action took place. Moreover, no attempt was made to subject the intracardial nodes to thorough morphological examination.

In the present research we attempted to correlate the alterations in cardiac activity and in its paraysmpathetic regulation, due to myocarditis, with the myocardial alterations and with changes in the morphology of intracardial ganglionic formations. An essential feature of these experiments should be the exclusion of any possibility of direct damage to intracardial nervous formations by the traumatizing agent employed, and this would permit of an approach to the solution of the second objective of our research, viz., the elucidation of the role of reflex mechanisms in origination of morphologically discernable alterations in the intracardial nervous apparatus.

This condition was satisfied by the technique already applied by us for the production of focal myocarditis, and consisting in the injection, through a fine needle, of 1.0-2.5 ml of physiological saline at 85-95° into the apical myocardium, thus producing a circumscribed focus of necrosis, followed by hemorrhage and inflammation.

EXPERIMENTAL METHODS

The experiments were performed on dogs. The right vagus nerve of dogs under morphine-ether-chloroform anesthesia was cut in the cervical region, and the peripheral segment was placed on an electrode connected with the secondary coil of an inductor, fed from a 4 v accumulator. In each experiment we first determined the threshold for the reaction of the heart, during 10-15 minutes, involving 5-6 stimulations, each of 10 seconds duration. Hot saline was then injected into the myocardium (for this purpose, the thorax was opened at the level of the fifth rib, for 3-5 minutes, with artificial respiration, and an incision was made in the pericardium), and 8-10 determinations of the threshold current were again made, over a period of 40 minutes to 1 hour. Recordings of the action of the heart were made simultaneously with electrocardiographic and blood pressure recordings. The duration of each experiment varied from 1 hour to 1 hour 30 minutes. After conclusion of the experiment the dogs were killed with an ether-chloroform mixture.

Fragments of apical myocardial tissue, taken from the traumatized region, were subjected to histological examination, performed under the supervision of Prof. A. A. Solovev, Corresponding Member of the Academy of Medical Sciences USSR.

The tissue sections, prepared on a freezing microtome, were stained with hematoxylin-eosin, and with Sudan III for fat.

Intracardial nodes were taken from the region of the interauricular septum, and were examined by Nissl's method, and also by silver impregnation according to Kampos. The hearts of 4 experimental animals was subjected to histological examination. The control material for these examinations was taken from 2 dogs, previously subjected to a dummy operation, involving opening of the thorax, with incision of the pleural and pericardial sacs, without damaging the myocardium, followed by stimulation of the vagus, repeated 12-15 times at 3-5 minute intervals, for 60-70 minutes. Control material was also taken from 2 dogs which had been subjected to the dummy operation alone.

As was noted in our previous papers, stimulation of the vagus nerves of animals with undamaged myocardium did not affect the threshold of the reaction of the heart.

EXPERIMENTAL RESULTS

The reactions of all the 4 dogs, whose hearts were later subjected to histological examination, to the injection of hot saline were of the same type.

During the first few minutes after damaging the myocardium the threshold for the reaction of the heart to vagus stimulation rose by 2-8 cm of the length of the induction coil, i.e., the transmission of impulses from the vagus nerves to the heart was considerably impeded. The frequency of the heart beat increased. Sporadic or grouped premature beats made their appearance. Brief attacks of paroxysmal ventricular tachycardia were seen less frequently, although alterations in the form of the ventricular complex of the sinus contractions of the heart were not noted. This is evidence that the necrotic foci produced in the myocardium were of small size. The blood pressure rose slightly in some cases, and fell slightly in others.

Histological examination showed the following.

In Dog No. 1 (experiment of February 25, 1951) the muscle fibers in the specimen taken from the region of injection of saline into the apical myocardium were separated. The space between them was filled with loose fibrous tissue, forming a more or less dense reticulum. This enclosed sporadic groups of erythrocytes, as well as sparsely scattered lymphoid cells, including some polymorphonuclears, either singly, or in small aggregates. In places, this reticulum resembles a fibrin network. Some of the meshes of the reticulum are formed from separated connective tissue fibers.

The muscle fibers at the margins of the lesion frequently contain pycnotic nuclei, and are in places converted into a homogeneous or finely granular mass (Figure 1).

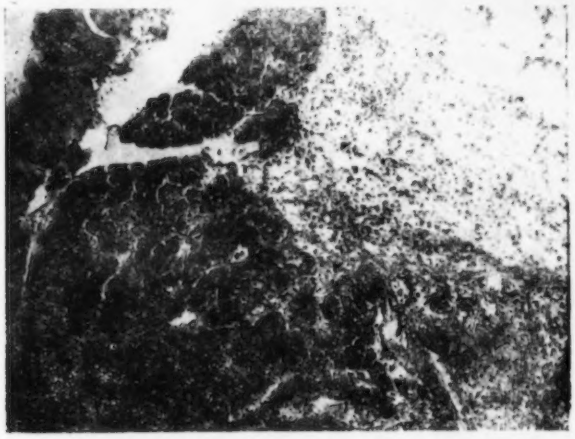


Fig. 1. Site of introduction of physiological saline into the apical region of the heart of Dog No. 1.

The blood vessels located in the immediate vicinity of the focus, as well as at a certain distance from it, were markedly engorged.

Staining for fat with Sudan III did not reveal fatty degeneration. The intracardial nodes contained nerve cells, with peripherally situated nuclei, and showing evidence of central chromatolysis. A few binuclear cells were seen.

Similar changes were seen in the other dogs (Nos. 2, 3, and 4).

Deviations from normal were not observed during the histological examination of heart muscle taken from dogs of the control series, subjected to the same operational trauma as the experimental group, and to vagus stimulation, but not given myocardial injections. The nerve cells of the intracardial nodes showed alterations characteristic of stimulation. These changes were not of uniform intensity for all the dogs of the control group.

Isolated nerve cells taken from dogs subjected to vagus stimulation contained peripherally situated chromatophilic substance, which formed a border around the margins of the cells, with peripheral displacement of the nucleus. Such cells were also encountered in dogs subjected to opening of the pleural and pericardial sacs only, without vagus stimulation, but the changes were of lesser degree.

A comparison of the nature of the alterations in the nerve cells of the experimental and the control animals shows that the differences are both quantitative and qualitative.

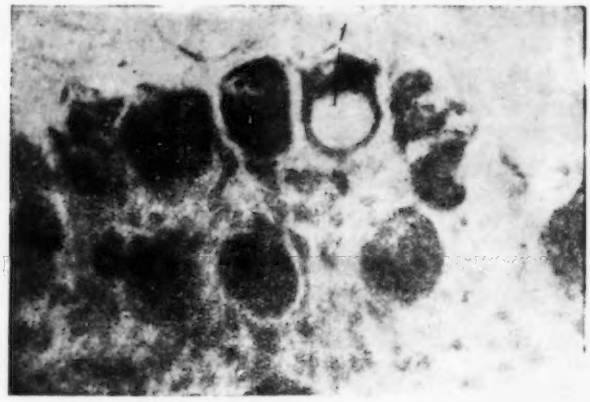


Fig. 2. A nervous node from the interauricular septum. 1) vacuolized nerve cell (Dog No. 1).

Whereas stimulation of the vagus, and opening of the thoracic cavity, are followed by appearance of evidences of stimulation of isolated neurones only, the superimposition of additional stimulation by inflicting circumscribed trauma to the myocardium is followed by the appearance of vacuolized nerve cells, and at hyperplasia of their processes, i.e., to more serious structural alterations (Figure 3).

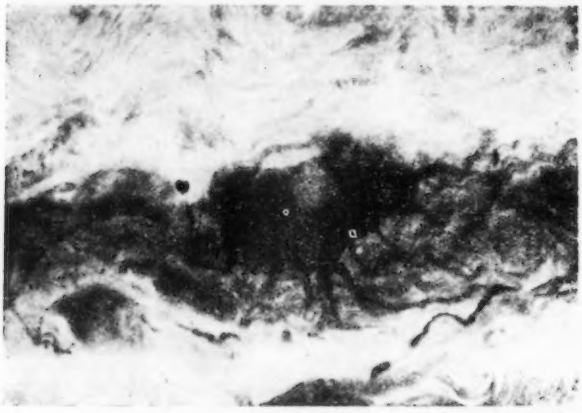


Fig. 3. A node from the interauricular septum. Hyperplasia of processes of a nerve cell (Dog No. 2).

Similar alterations in intracardial nodes were described by Yu. M. Lazovsky [3] in certain myocardial diseases, in particular in myocardial infarct.

The disturbances in transmission of impulses from the vagus nerves to the heart may to a large extent be attributed to these pathological dystrophic changes in nerve cell morphology, which are only encountered in the presence of lesions of the heart.

It may be concluded from the electrocardiographic findings, from the morphological study of the thermal lesions, which are seen to be sharply demarcated, and to be remote from the locations of the intracardial nervous apparatus, and from the rapid development of the changes in this apparatus, that these alterations are not ascribable to the direct action of the traumatizing agent, but that they arise reflexively, as a result of the powerful additional stimuli proceeding from the focal lesion.

The stimuli from the focal lesion may reach the intracardial nervous apparatus through existing reflex arcs, involving the second, intact vagus nerve, the intervertebral ganglions, and the sympathetic nerve. Of equal importance may be links of the axon reflex type, and this view is supported by A. A. Zubkov's comparative morphological studies [2]. Phenomena of the type of perielectrotonic remote action may also be of some significance [7].

LITERATURE CITED

[1] V. V. Zakusov, Farmakol i Toksikol., 17, No. 4, 3-9 (1954).

[2] A.A. Zubkov, Fiziol. Zhur. SSSR, 11, 229 (1939).

[3] Yu. M. Lazovsky, Klin. Med., 22, No. 9, 45-50 (1944).

[4] V. S. Livshits, Disease, Treatment, and Recovery,* pp. 105-122 Moscow, 1952.

[5] Ibid., Problems of Reactivity and Pathology,* pp. 123-127 Moscow, 1954.

[6] V. V. Frolkis, Byull. Eksptl. Biol. i Med, No. 3, pp. 21-24 (1954).

[7] I. A. Chernogorov, Physiological Nature of Heart Block,* Moscow, 1948.

ALTERATIONS IN PHAGOCYTIC ACTIVITY OF THE LEUCOCYTES OF HUMAN BLOOD DURING ANESTHESIA

Z. E. Matusis and V. V. Sherstobitova

From the Bacteriology Laboratory (Director: Senior Scientific Worker Z. E. Matusis), Gorky Institute of Restorative Surgery, Traumatology, and Orthopedics (Director: Assistant Professor M. G. Grigorev)

(Received March 16, 1956. Presented by V. N. Chernigovsky, Member Acad. Med. Sci. USSR)

Numerous papers have been published over the past few years on the effect of the nervous system on immunobiological processes, and, in particular, on the phagocytic reaction of leucocytes [5, 10, 11, 12, 13]. Most of the researches were performed on animals, and only a few [4, 9, 11] are based on observations of human subjects.

The present paper presents the results of observations of phagocytic activity of the leucocytes of 127 patients under ether or nitrous oxide anesthesia. The complement activity of the blood was also studied for some of the patients.

EXPERIMENTAL METHODS

Phagocytic activity and complement titer were determined in 110 patients before administration of the narcotic, during the stage of unconsciousness preceding the operation, and on the day following the operation. Of these patients, 81 underwent surgical operations for conditions such as contractures, ankyloses and skin defects, i.e., they were not suffering from inflammatory or septic conditions. The complement titer of the blood was determined by the same method as is used in the Wasserman reaction. The phagocytic activity of the leucocytes was assessed with respect to Staphylococcus aureus, strain 209. To a mixture of 0.02 ml of 3.8% sodium

* In Russian.

citrate solution and 0.08 ml of blood we added 0.05 ml of a staphylococcus suspension (containing 2×10^9 organisms per ml) in physiological saline, and the mixture was incubated at 37° for 20 minutes. A smear was then made (fixed with Nikiforov's mixture, stained with methylene blue), and counts were made on 5 smears, each of 20 leucocytes. A shift in the phagocytic number (number of cocci taken up per leucocyte) was taken as significant if it exceeded the initial (preanesthesia) value by more than 10%.

EXPERIMENTAL RESULTS

Observations on 25 patients showed that the complementary activity of the blood did not vary beyond the limits of accuracy of the determination, irrespective of the nature or the amount of the anesthetic used, and we did not proceed further with this aspect of the investigation.

Of 94 patients examined on the day following the operation, 57 had a phagocytic number lower than before administration of the anesthetic, 14 showed the same value, and 21 a higher one. Since these data represent not only the effects of anesthetization, but also of the surgical treatment, we shall not here discuss them.

An examination of the material showed that the variations in phagocytosis were not correlated with the nature of the anesthetic, or with the amount of it used, or with the existence of any septic process in the patient. Thus the phagocytic index for patients receiving nitrous oxide rose in 14, fell in 89, and was unchanged in 4 cases. The corresponding figures with ether anesthesia were 8, 42, and 3.

Rise in phagocytic index was observed in 19 patients not presenting septic complications, a fall in 57, and no change in 5 patients. The corresponding figures for patients with sepsis were 3, 42, and 2.

Blood was taken for the second examination when the patient was under anesthetic, shortly before the operation. In 21 patients, however, the blood samples were taken at the end of the stage of excitation, as the operations were begun before the patient was deeply unconscious; in 17 of these phagocytosis was higher than the initial value, in 2 lower, and unchanged for the remaining two. The other 89 patients were fully anesthetized at the time of blood sampling: increased phagocytosis was seen in only 5 patients of this group, and no change in 5, while the index was markedly lowered for the remaining 79 patients.

In connection with these findings, we made a special examination of a further 17 patients, with the object of elucidating the effect of the stage of excitation on the phagocytic activity of leucocytes, and of determining whether the lowered phagocytosis observed during anesthesia is not only a relative effect; the emotional state of the patient of the day of the operation, and while waiting for it, may have exerted a stimulatory effect of phagocytic activity, and the apparent fall during the action of the anesthetic may have been only a reversion to the normal level.

The phagocytic activity of these patients was twice determined during the week preceding the operation, a third time immediately before the operation, and twice while the patient was under the anesthetic, once at the height of the stage of excitation, and once during profound sleep. In 3 patients only was the phagocytic activity different (higher) from that found during the previous week. This is evidence of the incorrectness of the supposition that the phagocytic activity of these patients was at an abnormally high level when they entered the operating theater and the subsequent fall might have been no more than a return to the normal value.

The phagocytic index during the stage of excitation was raised in 10 of 17 patients, unchanged in 4, and lowered in 3 cases. During profound anesthetic sleep 15 of the 17 patients showed a definite lowering of the index, as compared with both the preanesthetic state and with that of excitation. In one case only was it raised, and in one case unchanged.

Apart from our observations on patients, we performed control experiments, with the object of ascertaining whether ether, at the dosage levels used, has any direct effect on leucocytes, leading to change in their phagocytic activity. Blood was placed in a hermetically sealed glass vessel, under an atmosphere containing a concentration of ether vapor (2.8 mg per ml) which would cause profound narcosis in the human. After 30 minutes of contact with the vapor we could not find any change in the phagocytic activity of the leucocytes.

It thus appears that ether or nitrous oxide anesthesia leads to a rise in the phagocytic activity of leucocytes during the stage of excitation, and to a fall during that of profound unconsciousness. The question arises as to how these central nervous system effects can be transmitted to the leucocytes, which have no direct connection with the nervous sytem.

The work of N. V. Puchkov, G. V. Golodets, and others [3, 6, 8] has shown that mediators can exert a significant action on the phagocytic activity of leucocytes: sympathicotropic substances stimulate phagocytosis, while vagotropic ones (acetylcholine) depress it. M. Ya. Mikhelson's experiments [7] showed that narcotic substances exert a powerful inhibitory action on cholinesterase, both in vivo and in vitro. It might be supposed that anesthetics cause a rise in the acetylcholine content of the tissues, and hence to an inhibition of phagocytosis. When the blood samples were taken during the stage of excitation, in which the tonus of the sympathetic system was raised, the increased production of sympathicotropic substances heightened the phagocytic activity of the leucocytes.

LITERATURE CITED

[1] G. V. Vygodchikov and O. V. Barykina, Zhur. Eksptl. Biol. i Med., 6, No. 17, 538-541 (1927).

[2] A. A. Gogochkina, Problems of Hypertensive Disease,* pp. 374-379, Gorky, 1951.

[3] I. N. Golovkova, G. V. Golodets, N. V. Puchkov, and S. M. Titova, Proceedings of the 7th All-Union Congress of Physiologists, Biochemists, and Pharmacologists,* pp. 305-308, Moscow, 1947.

[4] G. G. Golodets, Byull. Eksptl. Biol. i Med., 21, No. 1-2. 67-69 (1946).

[5] A. I. Karaev, G. Guseinov, and S. Ragimova, Izvest. Akad. Nauk SSSR, No. 7, 21-28 (1952).

[6] R. U. Lipshits, Voprosy Eksptl. Biol. i Med. No. 1, pp. 89-93 (1951).

[7] M. Ya. Mikhelson, Proceedings of the 7th All-Union Congress of Physiologists, Biochemists, and Pharmacologists,* pp.348-349, Moscow, 1947.

[8] N. V. Puchkov, Present-day Problems of General Pathology and Medicine,* pp. 123-127, Moscow, 1947.

[9] L. A. Stukanova, Peculiarities of the Phagocytic Reaction of Leucocytes and of the Erythematous Reaction of the Skin in Stuporous and Other Psychological States.* Thesis, Sverdlovsk, 1953.

[10] M. V. Troitskaya and A. I. Khokhlova, Zhur. Vysshei Nerv. Deyatel., 3, No. 5, 738-743 (1953).

[11] V. P. Tulchinskaya, R. O. Faitelberg, and I. V. Aplyak, Zhur. Mikrobiol., Epidemiol. i Immunobiol., No. 1, 23-26 (1954).

[12] M. A. Frolova and A. N. Shneerson, Zhur. Mikrobiol., Epidemiol. i Immunobiol., No. 5, 14-20 (1953).

[13] S. Metalnikov, Role du Systeme Nerveux et des Facteurs Biologiques et Physiques dans l'Immunite, Paris, 1934.

EFFECT OF BARBITURATES ON THE DIFFERENTIAL CELL COUNT OF PERIPHERAL BLOOD OF NORMAL ANIMALS AND IN EXPERIMENTAL ANEMIA

M. F. Runova

From the Pharmacological Laboratory (Director: Prof. V. I. Skvortsov, Member Acad. Med. Sci. USSR), Institute of Pharmacology and Chemotherapy (Director: Prof. P. G. Snyakin), Acad. Med. Sci. USSR

(Received June 16, 1955. Presented by V. I. Skvortsov, Member Acad. Med. Sci. USSR)

The present paper gives the results of an investigation into the effects of some bartiturate hypnotics on the differential cell count of peripheral blood. It is a continuation of earlier published researches [1].

*In Russian.

**Original Russian pagination. See C. B. Translation.

Effect of Noctal and Sonbutal taken over a long time on the differential cell count of peripheral blood

Groups of 10 mice each were given daily subcutaneous injections of 0.5% No tal or Sonbutal solutions, to which a few drops of 1 N NaOH had been added to dissolve the drug.

The dosage levels were such as to cause sleep lasting from 2 to 5 hours, viz., 0.5–0.7 mg of Noctal or 0.6–0.7 mg of Sonbutal per 10 g body weight.

The blood examinations (hemoglobin, erythrocytes, leucocytes, differential cell count, reticulocytes, thrombocytes) were done twice before the administration of barbiturates, and twice during administration. The mice were weighed before and during the experiment. The results of the experiments were subjected to biometrical evaluation, and are presented in Figure 1.

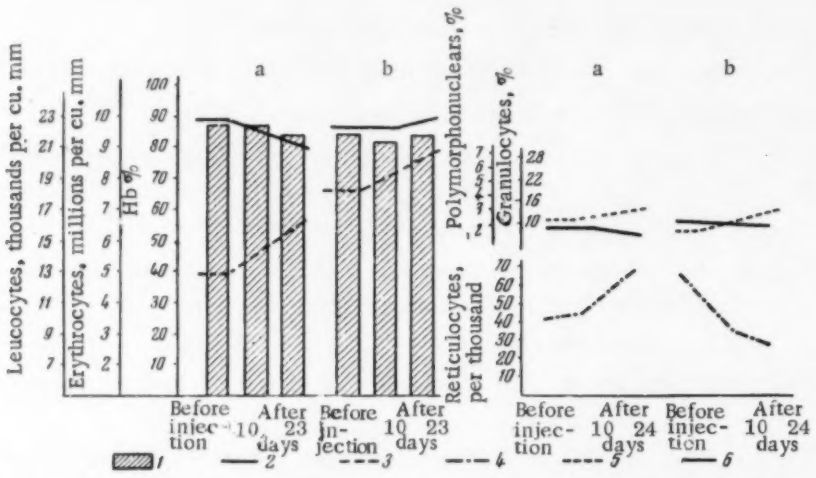


Fig. 1. Effect on the blood of prolonged administration of Noctal and Sonbutal to normal mice. a) Noctal; b) Sonbutal. 1) hemoglobin: 2) erythrocytes; 3) leucocytes; 4) reticulocytes; 5) granulocytes; 6) polymorphonuclears.

The only changes seen during prolonged administration of barbiturates were a slight reticulosis with Noctal, and a slight fall in reticulocyte count with Sonbutal.

A comparison of these results with those found earlier [1] for other barbiturates (Barbamyl, Nembutal, and Medinal) shows that the greatest effect on the blood is exerted by Barbamyl, prolonged administration of which lowers the hemoglobin and erythrocyte count, and raises the reticulocyte count.

Effect of a single dose of Noctal or Sonbutal on the differential cell count of peripheral blood

The animal material consisted of mice (10 for each drug), rabbits, and dogs.

The blood examinations were done before administering the drug, during profound sleep, and 3 and 24 hours after wakening.

The leucocyte count fell by an average of 52.7% during Noctal-induced sleep, and rose at the moment of wakening, although still remaining 36.6% below the initial value, which was found 24 hours after wakening. The differential cell count showed a small increase in granulocytes. The reticulocyte count did not vary.

The leucocyte count fell only very slightly during Sonbutal sleep, and was above the initial value by an average of 37% at the moment of wakening. The differential cell count showed a small rise in the proportion of granulocytes (Figure 2).

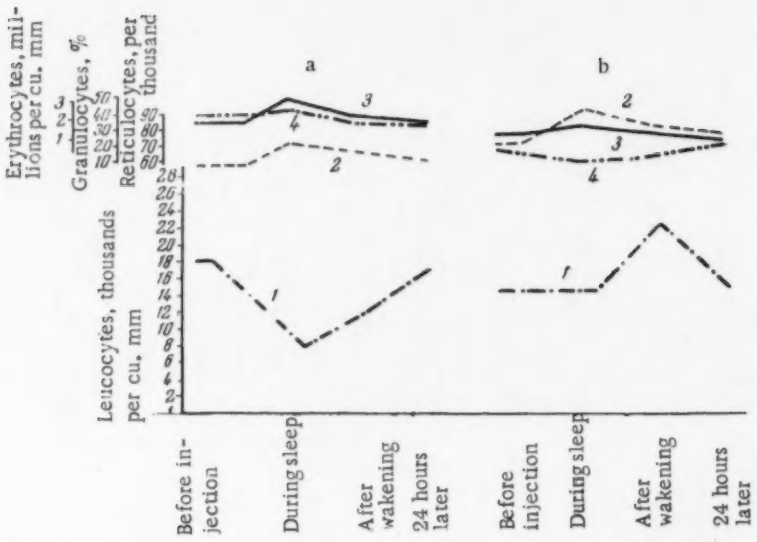


Fig. 2. Effect of sleep induced by Noctal and Sonbutal on blood cell morphology. a) Noctal, b) Sonbutal; 1) leucocytes; 2) granulocytes; 2) polymorphonuclears; 4) reticulocytes.

Comparison of these results with those of our earlier investigations [1] again show that Barbamyl has a more powerful effect than do the other barbiturates on the leucocyte count, which it depresses by an average of 63.5%.

Effect of Noctal and Sonbutal on peripheral blood cells in experimental anemia

The drugs were injected into groups of 10 mice each, leaving a control group of 10 mice. Anemia was produced by bleeding from a severed tail tip. The mice were bled daily for 4-5 days, withdrawing 0.3 ml of blood on each occasion. Over this period the hemoglobin content fell from 90% to an average of 35-40%, and the erythrocyte count from 9 to 4 million; anisocytosis and polychromatophilia, with Howell-Jolly bodies, were seen. The reticulocyte count rose from 30-40 to 240 per thousand. The leucocyte count fell in most of the animals, as did the thrombocyte count. Bleeding was then stopped, and the hypnotics were administered for 23 days, in the same dosage as before. The blood was examined before bleeding, after bleeding, but before administration of the drugs, and on the 7th and 23rd days of administration. The hemoglobin content and the reticulocyte and erythrocyte counts were determined.

Over this period of 23 days the blood picture reverted to normal in the control group and in those given Noctal or Sonbutal (Figure 3); the same findings were obtained with Medinal and Nembutal. As was found in our earlier experiments [1], anemia persisted in mice given Barbamyl, with only a slight rise in hemoglobin content and in erythrocyte count; the reticulocyte count remained high. The morphological changes also persisted, in the form of anisocytosis, polychromatophilia, and Howell-Jolly bodies.

It thus appears that prolonged administration of Noctal and Sonbutal does not cause blood changes in experimental animals, other than a reticulocytosis with No tal and a slight fall in reticulocyte count with Sonbutal; Medinal and Nembutal had also been found to have little effect on the blood.

The leucocyte count fell during barbiturate-induced sleep, to an extent proportional to the profoundness of the latter. The leucocyte count rises at wakening, gradually reverting to the initial value over the following 24 hours. The Arneth index shows a granulocytosis, with a slight shift to the left. The reticulocyte count falls. These effects are greatest for Barbamyl, less so for Noktal and Nembutal, and least for Medinal and Sonbutal.

It may be supposed that the changes found in the peripheral blood during sleep are due not only to redistribution of formed elements, but also to alterations in the hemopoietic processes, as is evidenced by the changes

in the Arneth index (granulocytosis, with a shift to the left, and a fall in the reticulocyte count).

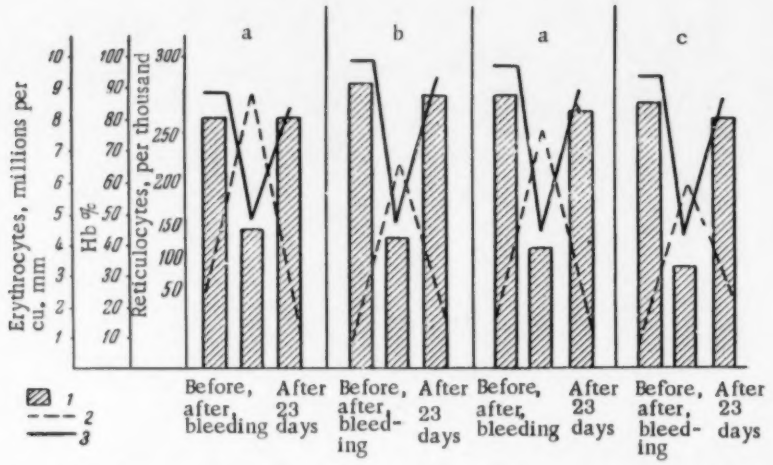


Fig. 3. Effects on the blood of prolonged administration of Noctal and Sonbutal to mice suffering from experimental anemia. a) controls; b) Noctal; c) Sonbutal; 1) hemoglobin; 2) erythrocytes; 3) reticulocytes.

In experimental anemia, produced by repeated bleeding, administration of Noctal and Sonbutal was, similarly to Medinal and Nembutal, without effect on the rate of recovery (the normal blood picture was restored equally soon in the experimental and control groups). Administration of Barbamyl, on the other hand, prolonged the time needed for recovery; the signs of anemia persisted over the 23 days of administration, at about the same level as immediately after bleeding.

LITERATURE CITED

[1] M. F. Runova, Farmakol. i Toksikol., 17, No. 6, 39-44 (1954).

UDDER RECEPTION IN COWS AND GOATS, AND THE COMPOSITION OF THE BLOOD

O. P. Belygina

From the Chair of Animal Physiology (Director: Honored Scientific Worker Prof. G. I. Azimov), All-Union Agricultural Correspondence Institute (Director: Assistant Professor P. P. Ipatov

(Received April 21, 1956. Presented by V. N. Chernigovsky, Member Acad. Med. Sci. USSR)

Alterations affecting the whole of the organism take place during lactation. Lactation, like any other process taking place in the organism, is under the control of the central nervous system. Our knowledge of how this nervous control of secretion and ejection of milk is achieved, and by which pathways, is, however, far from being complete.

A number of authors [2, 3, 4, 6] have indicated a reflex connection between the receptor apparatuses of the udders and the alimentary tract.

A study of the processes of secretion and excretion of milk, conducted in our laboratory on ruminants, revealed a rise in the dry content of the blood after milking the animals. A study of the blood morphology showed pronounced changes in the composition of blood after massage of the udders and milking.

V. N. Chernigovsky and A. Ya. Yaroshevsky [7] have given a number of examples of the effects of stimulation of the receptors of individual organs on the morphological composition of the blood. Thus, stimulation of gastric interoceptors causes alterations in red corpuscle and differential white cell counts. The findings of E. L. Kan [5], who stimulated cows' gastric interoceptors, are evidence of well-defined alterations in blood composition following such stimulation. A.Ya. Yaroshevsky's work [7] showed that alterations in the composition of the blood occur similarly after stimulation of dogs' gastric interoceptors.

The present paper presents preliminary results of experiments on the effects of stimulation of udder receptors on the blood picture of cows and goats.

EXPERIMENTAL METHODS

Blood examinations were performed on: a) lactating goats; b) not lactating animals, which had never lactated; c) lactating cows; d) heifers. The hemoglobin content, erythrocyte and leucocyte counts, and differential cell counts of the blood were determined.

EXPERIMENTAL RESULTS

Our first experiments, performed under laboratory conditions on goats, showed that massage of the udders and milking caused a considerable rise in the erythrocyte count, without affecting the hemoglobin content. In one of the experiments, a goat was milked once by its regular milker, and on another occasion by a different person, all other conditions being the same. As appears from Figures 1 and 2, massage and milking caused a

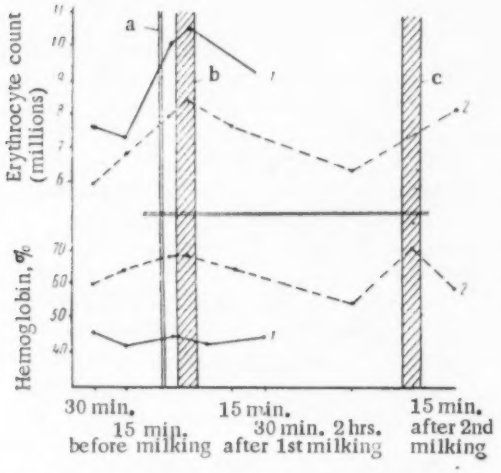


Fig. 1. Alterations in erythrocyte count and hemoglobin content of blood associated with milking. 1) Goat; 2) cow; a) entry of milkmaid; b) and c) massage and milking (first and second).

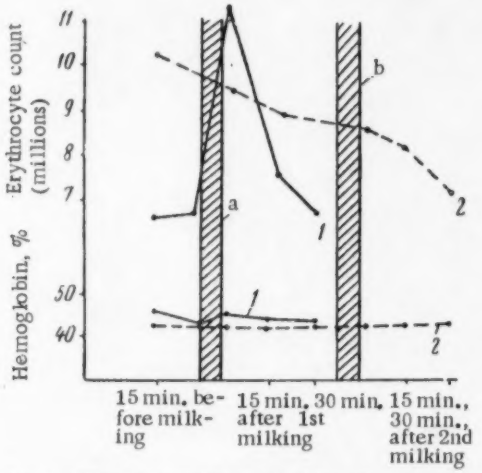


Fig. 2. Effect of condition of udder receptors on alterations in erythrocyte count and hemoglobin content. 1) Control cow; 2) cow with anesthetized udder; a) and b) massage and milking.

considerable rise in the erythrocyte count (from 7,280,000 to 10,950,000 in one case, and from 6,800,000 to 11,230,000 in the other), without any change in the hemoglobin content. It should be noted that the blood changes took place not only after massage and milking, but also after the action of other factors connected with these processes: the entry of the milkmaid caused as large changes in the blood picture as did the milking itself, which is evidence of the inception of a conditioned reflex arc (see Figure 1). The speed with which the

blood reaction appeared after milking is evidence of the part played by the central nervous system in producing the effect.

Experiments were performed on goats after previous anesthetization of the udders, by Magda's method. No blood changes were seen after massage and milking (see Figure 2). The high erythrocyte count found at the beginning of the experiment can be ascribed to the lumbar procaine block. It is known that lumbar procaine block causes a pronounced rise in the erythrocyte count [1]. The return to normal of the erythrocyte count coincided in our experiment with restoration of sensibility to the udders.

It may be concluded that the blood changes depend on stimulation of udder receptors; when these are excluded we could find no change in blood morphology.

The experiments on nonlactating goats gave a somewhat different result. Experiments were performed on Goat No. 16 before and after calving. This animal had never previously been milked. At the same time we examined the blood of a nonlactating goat, No. 8, which had been in milk the previous year. It was found that the goat which had never lactated did not show any blood changes after massage of the udders, but that these appeared after birth of the kid, and that they were identical with those found in all lactating animals after massage of the udders. The blood changes resulting from udder massage of Goat No. 8 were similar to those found in lactating animals, with the sole difference that they were of short duration.

Massage and milking are followed not only by changes in the erythrocyte count, but also in the differential white cell count: the lymphocyte count rises, and there is a slight fall in the neutrophil count. This reaction is of a short duration.

The changes observed in cows' blood after milking are presented in Figure 1 and in the Table. These changes are of the same sort as for goats, in that the erythrocyte count rises. But whereas in goats the hemoglobin content is unchanged, in cows massage and milking produce changes which vary parallel with the erythrocyte count. The lymphocyte count rises, and the neutrophil count falls slightly (Figure 3). The duration of this reaction is much less than that of the erythrocytosis.

As for goats which had never lactated, massage of the udders of heifers caused no changes in the blood.

Massage of the udders of cows, not followed by milking, evoked the same blood reaction as milking itself.

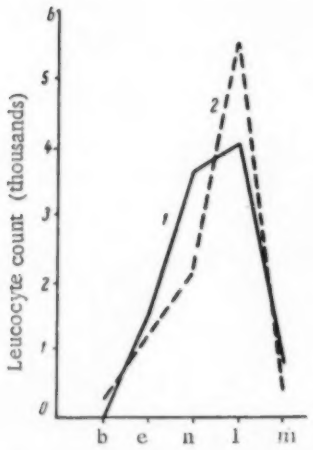


Fig. 3. Leucocyte count of cow's blood before and after milking 1) efore milking; 2) after milking; b) basophils; e) eosinophils; n) neutrophils; l) lymphocytes; m) monocytes.

Our experiments demonstrated the existence of nervous connections between udder receptors and the hemopoietic organs. It may be presumed that the bone marrow participates in this reaction, releasing erythrocytes of low hemoglobin content into the blood in response to stimulation of the udders. Further support is given to this presumption by the rise in reticulocyte count of the blood of goats after milking. The blood index (ratio of percentage hemoglobin content to the erythrocyte content in millions) falls steeply in goats after milking. It is evident that the mechanism of the nervous connections in cows is somewhat different to that in goats, since the index does not vary in cows after milking.

The changes in blood composition following the appearance of the milkmaid are evidence of the existence of a conditioned reflex mechanism. That these changes are not seen in animals which have never lactated, in response to massage of the udders, suggests that this reflex is established during the first lactation. Further research may reveal the pathways whereby the reaction is effected. The process of milking, and subsequently the preliminaries to milking alone, evoke a definite reaction in the composition of the blood. It may be concluded that the process of milking is of considerable significance to the organism as a whole.

TABLE

Effect of Milking on the Erythrocyte Count and Hemoglobin Content of Cows

When experiment performed	Name of cow							
	Mol		Gazel		Zenitka		Volzhanka	
	erythrocytes, millions	Hb, %	erythrocytes, millions	Hb %	erythrocytes, millions	Hb %	erythrocytes, millions	Hb %
Before milking	7.50	50	5.91	56	6.09	50	5.92	60
During Milking	8.59	60	7.20	60	7.89	60	8.67	69
30 minutes after milking	—	—	—	—	7.08	51	7.91	66
40 minutes after milking	7.86	51	6.72	52	—	—	—	—
1 hour 30 minutes after milking	6.77	—	6.24	51	6.01	53	6.18	54
During 2nd milking	8.62	60	7.19	61	7.08	59	7.36	68
25 minutes after milking	8.04	62	7.75	61	—	—	—	—
40 minutes after milking	—	—	—	—	—	—	8.50	59
1 hour after milking	—	—	—	—	6.28	50	—	—

LITERATURE CITED

[1] V. G. Vogralik, Nervous Trophism in the Theory and Practice of Medicine,* 1936.

[2] I. I. Grachev, Zhur. Obshchei Biol., 10, No. 4, 6 (1949).

[3] I. I. Grachev, Zhur. Obshchei Biol., 10, No. 5, 6 (1949).

[4] I. I. Grachev, Doklady Akad. Nauk SSSR, 78, No. 2, 6 (1951).

[5] E. L. Kan, Byull. Eksptl. Biol. i Med., No. 8 (1954).

[6] A. F. Orlov, Fiziol. Zhur. SSSR, 60, No. 1, 6 (1954).

[7] A. Ya. Yaroshevsky, Byull. Eksptl. Biol. i Med., 8, 8-12 (1954).

BLOOD CHANGES DURING PHYSIOLOGICAL SLEEP

M. I. Ulyanov

From the Pathophysiological Laboratory, Chief Military Hospital, Moscow

(Received September 16, 1955. Presented by V. N. Chernigovsky, Member Acad. Med. Sci. USSR)

Nocturnal sleep causes alterations in a number of the vegetative functions of the body. Variations in the cytomorphology of peripheral blood during sleep have been little investigated, although the study of hematological and other functional shifts taking place in the organism during sleep is necessary in the application of sleep therapy. The results of a study of the cytomorphology of peripheral blood during nocturnal physiological sleep will be reported in the present paper.

EXPERIMENTAL METHODS

The observations were carried out on 32 healthy men, aged from 21 to 23 years. In 22 of them two blood samples were taken; one before sleep, at about midnight, and one during sleep, at about 6 a.m. Four blood samples were taken from the remaining subjects: one before sleep, and three during the night, at 2-hourly

* In Russian.

intervals. The first sample was taken 30 minutes after the subject had gone to bed, but before he was asleep. Supper was taken 5-6 hours before taking the first sample. Sampling of sleeping subjects was done in such a way as to cause the least disturbance of sleep. Sleep was profound in most of the subjects, which was to be expected in view of their age and good physical condition. The sleep of 13 of the subjects was interrupted by the second sampling, while the other 19 were not fully roused by this operation. Our estimate of the depth of sleep was based chiefly on the general reaction of the subject to the sampling operation. Some indication of the depth of sleep was also derived from interrogation of the subject on the following day.

We applied the usual techniques of hematological investigation, slightly modifying the method for supravital staining of reticulocytes. A drop of blood was mixed in a paraffined watch-glass with an equal volume of physiological saline, and a small drop of a saturated alcoholic solution of brilliant cresol blue was added from a Pasteur pipette. The mixture wasmixed thoroughly, and kept in a damp chamber for 15-20 minutes, after which a smear was made. The granuloreticulo-filamentous substance of erythrocytes is stained sharply by this procedure. The smears were counterstained according to Romanovsky, for greater constrast.

EXPERIMENTAL RESULTS

A number of changes in the blood picture were found after 5-6 hours of nocturnal sleep. Taking the mean error of counting formed elements of blood as ±7% for leucocytes, ±2.5% for erythrocytes, and ±5% for thrombocytes, we observed a definite fall in leucocyte count in most of the subjects, on the average from 9,800 to 8,000 per cu. mm. An analysis of the absolute hemogram values showed that this decrease involved all of the white cells, with the exception of the eosinophils, both the proportion and the absolute number of which rose in most cases.

In the majority of subjects the erythrocyte count rose, by 3-26% of the waking value. The reticulocyte count rose parallel with this, and there was a shift to the left in the reticulocyte index. Hemoglobin content did not vary beyond the limits of accuracy of the method. The color index fell noticeably in most cases.

Hematocrit readings were taken for 17 subjects, and the mean erythrocyte volume was calculated. Both of these values rose during sleep (Table 1). It appeared from the experiments in which 4 blood samples were taken that these values may vary widely during sleep.

We also found qualitative and quantitative differences in the thrombocyte fraction. There was a slight thrombocytosis, together with an increase in the number of large forms of thrombocytes, diminished specific granulosity, and increase in the size of the hyaloplasm.

TABLE 1

Effect of Sleep on the Basic Red Blood Cell Indices (mean values for 17 subjects)

When samples taken	Hemoglobin		Erythrocytes, millions	Color index	Hematocrite, %	Mean corpuscular volume, cu. μ	Reticulocytes, %
	%	g per 100 ml					
Before sleep	78.4	12.5	4.77	0.86	36	75	5.5
During sleep	78.6	12.6	5.03	0.79	40	80	8.1

It thus appears that natural night sleep causes a number of quantitative and qualitative changes, involving all of the formed elements of peripheral blood. These alterations are apparently due not only to redistribution processes, but also to changes in the functional state of the blood-forming organs, at least in the sense of release of cells into the circulation (reticulocytosis). Bearing in mind the general tendency of hematological changes, it should be noted that some form of deviation from the normal basic indices was found in a number of individuals before sleep.

Thus, 9 subjects had a leucocyte count amounting to 11,000-12,000 per cu. mm., and 13 individuals showed thrombocytopenia, of the order of 90,000-100,000 blood platelets per cu. mm. A slight erythrocytopenia was seen in some cases. The result of sleep appeared to be to bring the hematological indices closer to the normal values.

This normalization of the blood morphology is most clearly evident in profound sleep.

There are sufficient grounds for believing that these hematological alterations taking place during sleep are to a considerable extent a result of changes in the functional state of the higher levels of the central nervous system, inasmuch as the physiological basis of sleep lies in an inhibitory process arising in the cerebral cortex, and spreading to the lower levels. In connection with this, we thought it would be instructive to compare the nature of the hematological changes found in persons whose sleep was calm and profound (19 subjects) with those of a group of 13 whose sleep was superficial and uneasy. The mean values found in these two groups are presented in Table 2. It appears that the total leucocyte count in individuals whose sleep was peaceful and deep fell, as a result of a diminution in the numbers of all the species of white cells, with the exception of eosinophils, the absolute number of which rose in the great majority of cases. A fall in the neutrophil count was seen, with a decrease in the nuclear index of the neutrophil shift of 0.1 to 0.08, on the average. This fall in leucocyte count was not observed in the group of 13 whose sleep was shallow and uneasy, and in 4 of these subjects the leucocyte count even rose by 9-28%. The neutrophil count was raised, with an increase in the nuclear index of the neutrophil shift of 0.06 to 0.09, and the eosinophil count tended to fall. The erythrocyte and thrombocyte counts rose, although to a smaller extent than during profound sleep. The reticulocyte reaction was also somewhat more pronounced.

The findings for the 10 subjects whose blood was sampled 4 times during the night were in conformity with those presented above.

TABLE 2

Effect of the Type of Nocturnal Sleep on the Alterations in the Absolute Values of the Hematological Indices (as percentages of the initial values)

Type of sleep	Number of persons in group	Erythrocytes	Reticulocytes	Thrombocytes	Leucocytes	Leucocyte formula				
						eosinophiles	juvenile + mast cells	polymorphonuclear	lymphocytes	monocytes
Calm, profound	19	+7	+45	+26	−30	+34	−19	−27	−38	−33
Uneasy, shallow	13	+5	+91	+8	+1	−6	+62	+6	−12	−9

It thus appears that the changes in the cytomorphological composition of the peripheral blood taking place during sleep are closely bound up with the nervous processes which control sleep, as a definite functional state of the organism. The lowering of a number of hematological indices during profound sleep may be explained as being a consequence of irradiation of the inhbitory process to subcortical formations, which are concerned in the regulation of the composition of the blood. In persons whose sleep is shallow the excitability of the subcortical formations is enhanced, owing to the weakening of the regulating effect of the cerebral cortex, and also because of positive induction; as a result certain hematological indices increase. The absence of parallellism in the variations in esosinophil count and of other kinds of leucocytes during sleep, and the opposite direction of the variations in erythrocyte and leucocyte counts, are evidence of the complex nature of the processes taking place in the subcortical formations concerned in the regulation of blood composition.

TECHNIQUE OF DETERMINING THE RATE OF PROPAGATION OF THE PULSE WAVE IN HUMANS AND ANIMALS

V. E. Busygin and Yu. G. Nefedov

(Received January 30, 1956. Presented by A. I. Nesterov, Member Acad. Med. Sci. USSR)

Valuable information about the function of the peripheral vascular system under clinical and experimental conditions may be derived from a study of the dynamics of the rate of propagation of the pulse wave.

The subject has been very little investigated up till now, however, chiefly because of the lack of simple and trustworthy apparatus for its measurement.

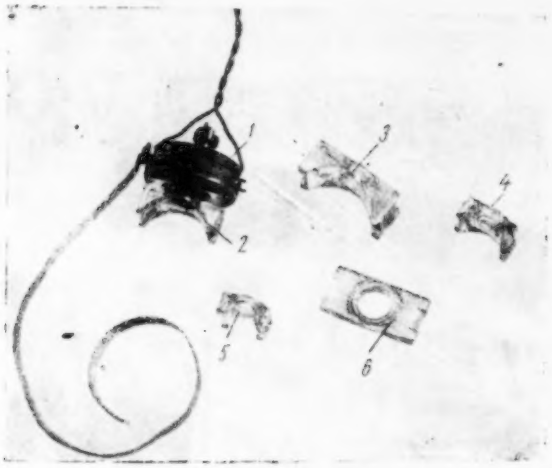


Fig. 1. Pulse recorder, with elastic tape for attachment, and a set of applicators for fixing the instrument. 1) ulse recorder; 2), 3) and 4) applicators for positioning the counter to the extremities: 5) applicator for the carotid artery, exteriorized in a skin tag; 6) for application to the chest.

We used pulse recorded designed by us, without amplifying arrangements [1], which provided a simple and readily available means of measuring the rate of propagation of the pulse wave. In order to register the time taken for a pulse wave to travel through a given section of the arterial system, or of an artery, tracings of the pulse wave were recorded at two or more points at various distances from the heart, with simultaneous recordings of a time marker. From the pulse tracings and the recordings of the time marker we derived the time taken for a pulse wave to travel between the selected points of the arterial system. Knowing this time, as well as the distance between the two reference points from which the pulse was recorded, we could calculate the velocity of propagation of the pulse wave over the given section of the arterial system.

The equipment for recording the rate of propagation of the pulse wave consisted of pulse counters, control panels, an electromagnetic oscillograph, and a time marker.

The pulse recorder is an ordinary carbon powder laryngophone, fitted with a cork disk fastened to a membrane. The recorder is fixed to the site where the pulse is to be measured. The way in which this is achieved depends on the location where the measurement is being made (Figures 1 and 2).

The control panel consists of a 4.5 volt battery (as used for a pocket torch), a resistance, from 400-700 ohms, and a switch. The circuit is shown diagrammatically in Figure 3.

The time marker, recording at 0.1 second intervals, consists basically of an electric motor, Type SD-60 (a synchronous electric motor, giving 60 r.p.m.), to the spindle of which is fastened an ebonite disk fitted with 10 brass contacts, with a fixed contact attached to the casing of the motor. Other designs of time marker may also be used. The electromagnetic oscillograph was of the usual design, with vibrators of a sensitivity of 5-10 mm per m. amp. The speed of travel of the photosensitive recording paper used for registration of pulse waves should be about 50-70 mm per second.

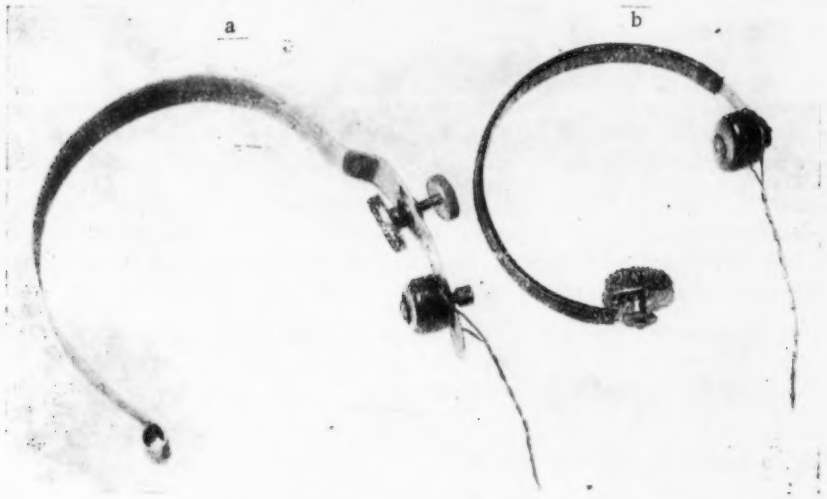


Fig. 2. Pulse recorders with a spring clamp, for registering pulse waves in human subjects. a) emporal artery; b) carotid artery.

The pulse recorders are fastened to the desired location, and are connected through the control panel to the vibrators of the oscillograph. The time marker is switched on in the same way, in connection with the corresponding vibrator (see Figure 3).

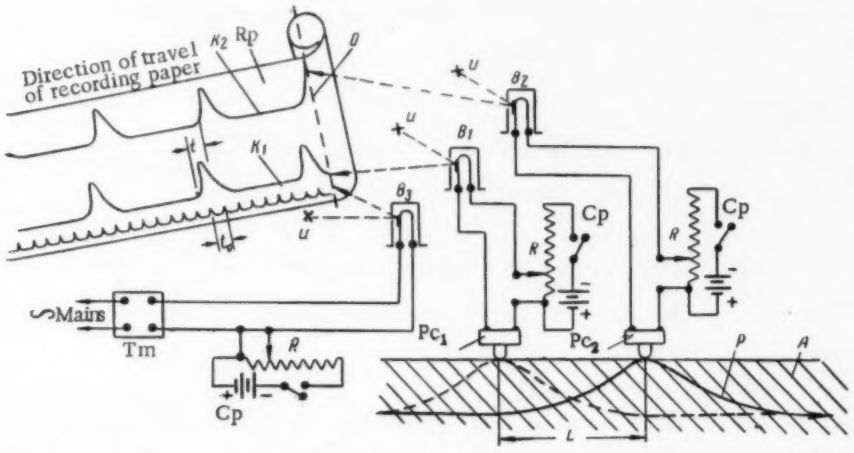


Fig. 3. Diagrammatic representation of registration of rate of propagation of the pulse wave. A) Artery; P) pulse wave; Pc_1) and Pc_2) pulse recorders; L) distance between pulse counters along an artery; Cp) control panel; R) variable resistance, for measuring the amplitude of tracings; B_1, B_2, B_3) vibrators; u) light source; Rp) recording paper; K_1 and K_2) tracings of pulse waves; Tm) time marker; t_0) time interval; t) time needed for propagation of pulse wave from one to the other counter.

Recording the rate of propagation of the pulse wave consists of the following. The pulse wave P proceeding along the artery A acts successively on the pulse recorders Pc_1 and Pc_2, changing their electrical resistance. The passage of the pulse wave along the artery A is recorded, as tracings K_1 and K_2 on the photosensitive paper, with the aid of the corresponding vibrators B_1 and B_2. At the same time, the time marker Tm records time intervals (0.1 seconds) on the same paper.

The rate of propagation of the pulse wave is derived from the tracings by the following calculation:

$$v_p = \frac{L}{t},$$

where v_p is the velocity of propagation of the wave, in milliseconds, L is the distance between the two counters, along the artery, in meters, $\underline{t}$ is the distance, expressed as seconds, between the beginning of an upward wave in tracing K_1 and of the corresponding wave in K_2, read from the number of time intervals $t_0 = 0.1$ seconds.

Before beginning the recording, the vibrators should be so adjusted that the spot of reflected light thrown on the paper by each of them is on the same vertical line, perpendicular to its length. If this is not done, the ordinates of the pulse tracings will be shifted, relatively to each other, which would lead to considerable error in calculating the absolute value of the speed of propagation of the pulse wave.

Care should also be taken that the pulse waves recorded are of about the same amplitude, as otherwise it may be difficult to establish accurately the beginning of the upward part of the pulse waves. The amplitudes are regulated by adjusting the variable resistances in the control panels.

Greater accuracy is achieved by making 3-4 readings of $\underline{t}$, at different points of tracings K_1 and K_2, and using the arithmetic mean for the calculation.

Pulse wave tracings may be taken from human subjects, using the radial, iliac, carotid, or temporal arteries, as well as the digital arteries of the hands and feet; the heart beat can also be recorded. It is thus possible to determine the rate of propagation of the pulse wave separately for different sections of the arterial system, or, if an oscillograph with a large number of vibrators is available, simultaneously for the whole system.

For animals, we usually determine the rate of propagation of the pulse wave along the section: origin of the aorta (from recordings of the heart beat or from the electrocardiogram) – anterior calcaneal artery.

LITERATURE CITED

[1] Yu. G. Nefedov, V. E. Busygin and S. V. Levinsky, Byull. Eksptl. Biol. i Med., No. 5,(1953).

METHOD FOR DETERMINING THE CIRCULATING BLOOD VOLUME, FOR EXPERIMENTAL PURPOSES

Yu. G. Nefedov (Moscow)

(Received January 30, 1956. Presented by A. I. Nesterov, Member Acad. Med. Sci. USSR)

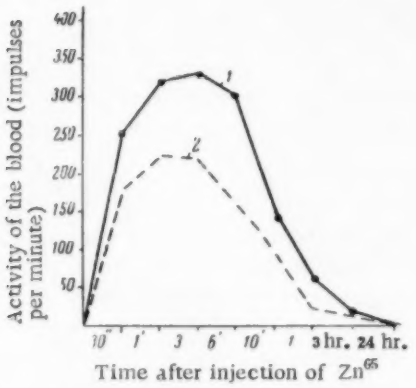
The circulating blood volume is one of the basic indicators of the condition of the hemodynamic function of the cardiovascular system. This volume is usually derived from measurements of the dilution of dyes introduced into the blood stream, usually Congo red or Evans blue. This technique cannot, however, be applied to the evaluation of circulating blood volume regularly over a prolonged period. The measurements cannot be repeated at shorter intervals than 6-7 days, since the dyes are retained in the blood for a long time.

There are also a number of methods for determination of blood volume based on gas analysis, but they are very time-consuming, and unsuitable for the execution of mass experiments.

A method for the determination of circulating blood volume was published by Wasserman, Joe, and Rashkof in 1951, depending on the use of radioactive phosphorus (P^{32}). Blood is taken from an animal, centrifuged, and the erythrocytes are suspended in a solution containing P^{32}. After a certain time the erythrocytes are separated and washed free of the solution, the specific activity of the packed cells is measured, and the cells are reintroduced into the blood stream, after suspension of an aliquot in physiological saline. Blood samples are then taken, and the circulating blood volume is evaluated from the degree of dilution of the labeled erythrocytes. This method is laborious and time-consuming. Morover, the lebeled erythrocytes persist in the blood stream for a long time, which interferes with repetition of the procedure.

It appeared to us that the procedure would be simplified if a solution of some radioactive isotope were to be introduced into the blood stream, instead of labeled cells. Our experiments showed that a radioactive isotope of zinc, Zn^{65}, is applicable to the determination of circulating blood volume. Radioactive isotopes of other elements, such as phosphorus, iron and sodium are very rapidly eliminated from the blood stream after injection of small indicator quantities. Thus the amount of radiophosphorus found in blood samples 1-2 minutes after its introduction is so small that the circulating blood volume based on calculations of its apparent dilution is impossibly large. Radioactive zinc, introduced in indicator quantities, is retained in the blood stream for a much longer time. This permits the derivation of circulating blood volume from the dilution of injected Zn^{65}.

A number of experiments were performed in order to ascertain the optimum times of sampling after injection of radioactive zinc. The results found for activity of samples of blood taken at different times after injection of Zn^{65} into an ear vein of a rabbit are shown in the Figure.



Activity of blood samples taken at various times after injection of Zn^{65}. 1) Iterations in activity of 1 cc of blood after injection of 1 cc of Zn^{65} solution of an activity of 49,400 impulses per minute; 2) the same, after injection two days after the previous experiments of 1 cc of Zn^{65} solution of activity 34,509 impulses per minute, into the same rabbit.

As appears from the Figure, the highest Zn^{65} contents are found in blood samples taken 1-3 minutes after injection. The activity of the blood falls considerably after 6 minutes, and it follows that radioactive zinc is being eliminated from the blood stream, and is practically undetectable after 24 hours. Blood samples should therefore be taken between 1 and 3 minutes after injection of the Zn^{65} solution. We usually took a blood sample 2 minutes after the injection. This gives enough time for the thorough and uniform mixing of the injected solution with the blood, as is shown by the close agreement between the values obtained for circulating blood volume using our method, and using other generally approved methods.

For determining the circulating blood volume of rabbits we usually took an indicator solution containing 30-40 μ curies of Zn^{65} per cc, while the solution for dogs contained 80-100 μ curies of Zn^{65} per cc.

The procedure for determining the circulating blood volume of rabbits consists of the following. One cc of Zn^{65} solution is injected into an ear vein.

The activity of 1 cc of solution is determined before every injection. For this purpose 0.01 cc of solution is spread by means of a micropipette on a slide, and its activity is determined, in impulses per minute. The background count is subtracted from that found for 0.01 cc of solution, and the result is multiplied by 100 to give the value for 1 cc.

Two minutes after injecting the solution 0.5–1 cc of blood is withdrawn from a vein of the other ear. In repeating the experiments it is necessary to inject solution and withdraw blood from the same ear as before. An even smear of 0.1 cc of blood, measured from a micropipette, is made on a slide, covering an accurately delimited area (we usually took an area of 1 × 4 cm), marked on the slide with a glass pencil.

Two such smears are made, the activity of each of them is measured, and the mean value is taken for calculating the activity of 1 cc of blood. The activity found for 1 cc of Zn^{65} solution is divided by the calculated activity for 1 cc of blood. The quotient gives the volume of circulating blood:

$$M = \frac{A}{a},$$

where M is the circulating blood volume in cc, A is the activity of the injected solution of Zn^{65}, in impulses per minute, and a is the activity of 1 cc of blood, in impulses per minute.

The following illustrates the calculation of the circulating blood volume of a rabbit, using the data presented in the Figure. The calculation is based on the activity of the blood sample taken 3 minutes after injection of Zn^{65} solution.

The data are: 0.01 cc of Zn^{65} solution gave 552 impulses per minute, and the background count was 58 impulses per minute, giving an activity for 1 cc of Zn^{65} solution $(552-58) \cdot 100 = 49{,}400$ impulses per minute; 0.1 cc of blood gave 93 impulses per minute, at a background count of 60 impulses per minute, giving an activity for 1 cc of blood of $(93-60) \cdot 10 = 330$ impulses per minute.

The circulating blood volume is:

$$M = \frac{A}{a} = \frac{49{,}400}{330} = 147.7 \text{ cc} \sim 150 \text{ cc}.$$

In repeating experiments on the same animal a blood sample was, for greater accuracy, taken before injection of radioactive zinc, and a smear was made of 0.1 cc of the blood; the activity of the smear served as the background count (it was usually identical with that of the counter itself). In most cases the activity of the pre-injection blood was zero.

The circulating blood volume of dogs is determined in the same way as for rabbits, except that injection of solution and taking of blood samples were performed on veins of the hind legs.

Determinations performed on large groups of animals gave values for circulating blood volume of 145-220 cc for rabbits, i.e., 5-6% of body weight; for dogs, the corresponding figures were 733-910 cc and 5-5.9%.

Our method determining circulating blood volume is simple, takes very little time, and provides the possibility of determining this important indicator of the state of the hemodynamic function of the cardiovascular system at daily intervals, and hence of studying the dynamics of changes in circulating blood volume.

PHOTOGRAPHIC METHOD OF STUDY OF THE VASCULAR PERMEABILITY OF THE HEMATO-OPHTHALMICBARRIER AND THE SKIN

N. I. Arlashchenko (Moscow)

(Received November 27, 1955. Presented by V. N. Chernigovsky, Member Acad. Med. Sci. USSR)

The permeability of the vessels of the anterior portion of the eye has been studied either by registering entry of dyes into the anterior chamber, or by withdrawing aqueous humor and determining its optical density [1]. Amsler and Huber [2] have proposed a visual method, depending on determination of the amount of fluorescein entering the anterior chamber; the eye of the patient was examined with the aid of a split lamp. By varying the resistance in the lamp circuit the intensity of the light could be adjusted until the green fluorescence of the fluorescein was no longer discernable. The strength of current at this moment was proportional to the reciprocal of the fluorescein concentration in the aqueous humor. Kurt Lange and Linn Boyd [3] have devized a photoelectric method, for the study of the permeability of skin. Fluoresceinwas injected intravenously, the skin was

illuminated with a blue light, and the intensity of fluorescence was determined with the aid of a photoelectric cell. It has not yet been possible, however, to apply this method to the detection of the entry of the dye into the anterior chamber, because of the extreme difficulty of excluding the brilliant fluorescence of the eyelids and sclera from the instrument.

Fig. 1. Emergence of fluorescein into the anterior chamber of the eye. a) before injuring the Gasserian ganglion; b) 3 hours later; c) 48 hours after.

The above-described methods of registering emergence of dye into the anterior chamber of the eye are either insufficiently objective, or else they do not permit of a dynamic study of variations in the permeability of the hemato-ophthalmic barrier. For this reason we attempted to investigate the permeability of the blood vessels of the anterior portion of the eye by photographic registration of the intensity of fluroescence of fluorescein penetrating into the anterior chamber after intravenous injection into an animal (at a dosage level of 20 mg per kg body weight). The dye solution was made up as a 5% solution in saturated sodium bicarbonate.

A number of workers have used ultraviolet light from a PRK-4 lamp, with a Wood's filter, for exciting luminescence of fluorescein. It has been found, however, that this excites luminescence of the sclera and the crystalline lens of the eye, and this interferes with the quantitative evaluation of the intensity of fluorescence of the dye entering the anterior chamber. We therefore used a point-source flashlight 275 watt bulb, with a double thickness of SS-5 filter, which has maximum transmission for light of wave length 4250 A. A stabilized beam of light was directed on a rabbit's eye, at an angle of 45°. A Zenith camera was fixed rigidly at an angle of 90° to the beam of light, with the objective situated at a distance of more than 7 cm from the film, which made it possible to photograph the rabbit's eye at a short distance from it. Photographs of the emergence of fluorescein into the anterior chamber, as shown by the fluorescence excited by the blue light, were taken on ordinary Isopanchromatic film at fixed intervals of time after injection of the dye. Blue light reflected from the surface of the eye was absorbed by a yellow ZhS-18 filter.

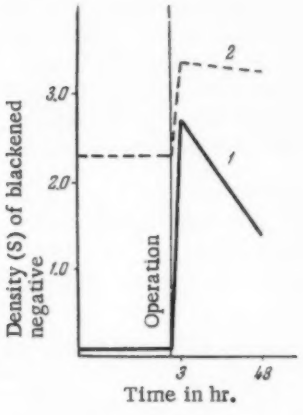


Fig. 2. Emergence of fluorescein into the anterior chamber of the eye. Derived from photodensity measurements of photographs. 1) Permeability of vessels of the anterior portion of the eye; 2) permeability of the skin.

In order to obtain comparable results, the photographs were taken on films of equal sensitivity, with a constant exposure time, measured by the strokes of an electric metronome. The films were developed under standard conditions with the developer solutions at the same temperature.

The density of the negative was measured with the aid of a MF-4 microphotometer, i.e., we measured the density S of the blackened parts of the film representing the image of the anterior chamber of the eye. These measurements were made at a number of points within the area concerned, as the intensity of fluorescence in the anterior chamber is not uniformly distributed. The mean values of S were then calculated, and served as a measure of the permeability of the vessels of the anterior portion of the eye to fluorescein. The values of S for standard solutions of fluorescein were obtained in an analogous manner, giving a factor for conversion of S values to percentage contents of fluorescein in the aqueous humor.

The proposed procedure for registering emergence of fluorescein into the anterior chamber allows us to follow

objectively the effects of various factors exerted on an intact animal on the permeability of the hemato-ophthalmic barrier. The same photographs can also serve for the study of the permeability of skin vessels; the values of S are determined for the hairless or almost hairless skin of the eyelids or around the eyes.

Figure 1 illustrates the course of change in permeability, as shown by photographs of fluorescence of a rabbit's eye, taken (a) a day before the Gasserian ganglion had been injured, (b) 3 hours later, and (c) 48 hours later. The photographs were taken 15 minutes after intravenous injection of fluorescein. Figure 2 presents the results of photometric treatment of the negatives. The degree of blackening of the relevant part of the negative (S) is expressed as the logarithm of the ratio of the intensity of light passing through the background parts of the negative to that passing through the exposed part.

LITERATURE CITED

[1] T. K. Dzharakyan, Fiziol. Zhur. SSSR, 32, No. 4, 464-477 (1946).

[2] M. Amsler and A. Huber, Arch. f. Ophth., 1949, Bd. 149, S. 578.

[3] K. Lange and L. J. Boyd, Arch. of Intern. Med., 1944, Vol. 74, pp. 175-184.

INTERPRETATION OF HUMAN PLETHYSMOGRAPHIC DATA

E. N. Kondratev (Moscow)

(Received April 23, 1956. Presented by A. N. Nesterov, Member Acad. Med. Sci. USSR)

The shortcomings of the techniques employed in the plethysmographic examination of human subjects emerge clearly from a study of the subject.

In particular, insufficient attention has been paid to the nature, magnitude, and frequency of distortions of the plethysmograms, due to movements of the subject. V. V. Yakovlev has shown, in experiments on dogs, that effects interpreted as plethysmographic reactions were in reality due to tonic contractions of the muscles of the extremity.

O. Bruns [12] has reported similar occurrences. It appeared that the subject (plethysmography of the arm) is usually unaware of contractions of his muscles such as can give a spurious vascular effect. From a comparison of plethysmographic tracings with those obtained as a result of muscle contractions registered in response to various stimuli (flexing and straightening the foot, pain, heat, cold), O. Bruns came to the conclusion that the use of plethysmography is justified only for the study of temperature effects, and even then only after special training of the subjects, who were enabled, by means of a separate device, to become conscious of "unconscious" movements of the arms, and to control them.

It should be noted that O. Bruns' observations were made chiefly with the aid of rubber bulbs in contact with the arm, and connected with the registering system (he makes a passing mention of the use of a galvanometer for this purpose, by one of his co-workers). It follows that some of the weaker movements, which might have had a distorting effect on the plethysmograms, were not recorded.

The object of the present research was to ascertain the effect of weak muscular contractions on a plethysmogram, obtained by one of the present-day techniques.

EXPERIMENTAL METHODS

Plethysmographic records were taken from the terminal phalanx of a finger of the left hand of 22 subjects, by the method of pneumatic finger plethysmography.

A glass cap is placed on the phalanx, and is connected by a rubber tube with a special micromanometer (1), fitted with a small mirror for photoregistration of the smallest pressure changes in the system (the micromanometer was mounted in the place of the vibrator of the oscillograph.

Slight movements of the finger were recorded by means of a flexible carbon powder rheostatic detector (1), attached to the finger and to the glass cap by strips of plaster (Figure 1).

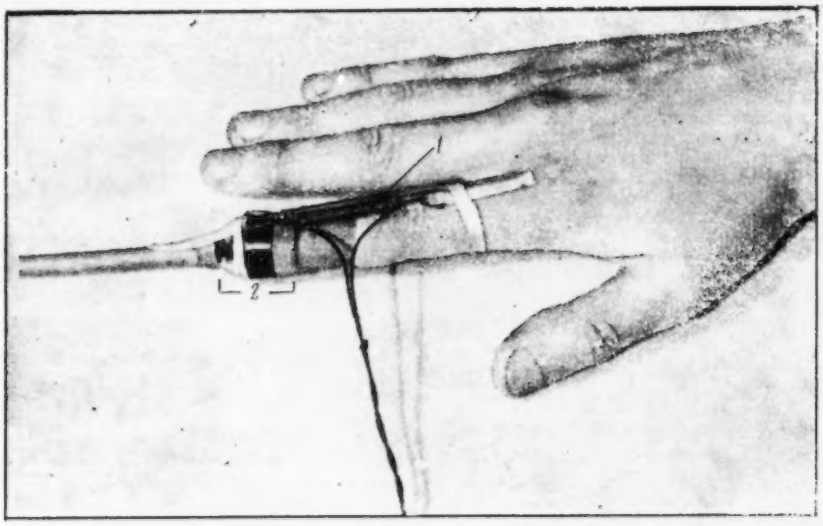


Fig. 1. Attachment of a carbon powder counter to a finger, for recording its movements. 1) Carbon powder counter; 2) glass cap.

The detector was connected in series with the oscillograph in such a way that its extension raised the tracing on the photosensitive paper, and its shortening lowered it. The detector and the vibrator were fed from a 3.2 v battery, through a potentiometer of resistance 450-600 ohms.

The sensitivity of the counter was sufficient to register slight, almost imperceptible movements of the finger. In many cases the finger movements record showed fluctuations due to the pulse, evidently transmitted through the skin of the finger to the detector. Respiratory movements were registered by means of a slightly modified laryngophone receiver fixed to the chest by means of elastic tape, and connected with the input of the oscillograph.

The recordings were taken continuously for 11 minutes for each experiment. A yellow and a red lamp (6 v; 7.5w) were switched on in succession for 2 seconds at $1^1/_2$ minute intervals.

The subjects were examined in this way for 3 days in succession, and they were told at the beginning of each experiment to lie still, and not to move their fingers.

EXPERIMENTAL RESULTS

It appeared that the number of "involuntary" movements of the fingers varied considerably for a given subject on different days. On occasions, there were several such movements per minute of registration.

In some cases the occurrence of finger movements could be detected from the nature of the distortions of the plethysmogram (Figure 2, a). In the case shown in Figure 3, however, it would not have been clear, except from the recordings of finger movements, whether the steep decline of the plethysmographic tracing (after a deep inspiration) is primarily a vascular reaction, or whether it was due to a finger movement, as happened a few seconds before. Other distortions of plethysmographic tracings due to muscle contractions are less frequently encountered, such as spikes (Figure 2, c), slight dips in the curves following finger movements (Figure 2, b), in which cases it would not be possible, without a recording of finger movements, to know whether and when the latter took place, on the basis of the plethysmogram alone. In some cases such smooth dips in the plethysmographic tracings undergo distortion as a result of previous abrupt finger movements (Figure 2, d).

An additional visual check showed that the spike effect can be produced by a slight voluntary movement of the finger, taking place a little earlier. Distortions of the tracings can also be produced by slight, continuous pressure, almost imperceptible to the eye, of the terminal phalanx into the cap; in such cases deviations of the finger movement control curve may still be seen. Finally, there are cases in which slight movements of the finger do not affect the plethysmographic tracing.

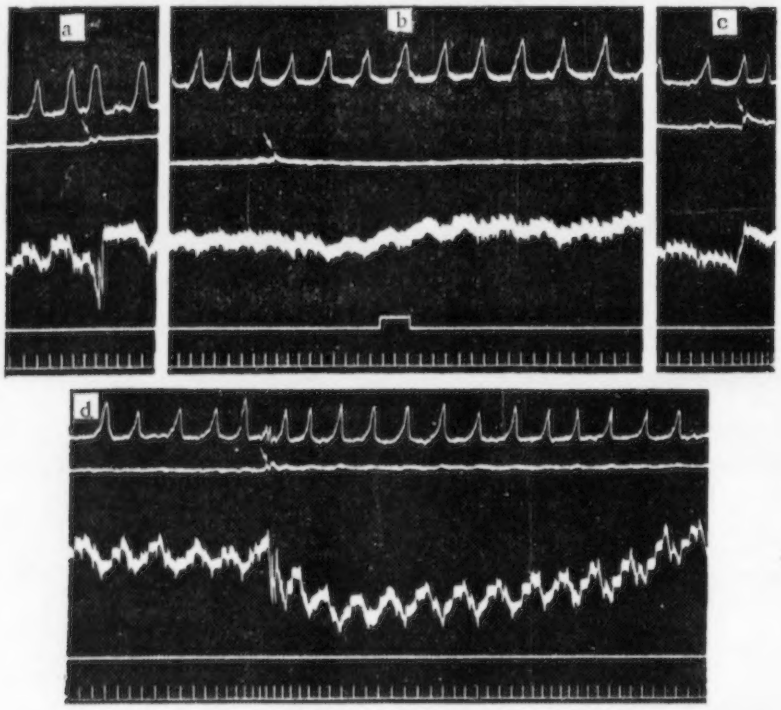


Fig. 2. Distortions of the plethysmogram due to "involuntary" movements of the finger. Explanation of tracings (from above down): respiration; record of finger movements; plethysmogram; signal showing when lamps switched on; time signals (1 second). Separate experiments.

We were not, as a rule, able to perceive finger movements which might be ascribed to the motor component of the orientational reaction; this may possibly be due to the low intensity of the light stimuli.

The finger movements may arise in consequence of various interoceptor effects [3, 9], or of irradiation of impulses from the respiratory center to the motor areas [2, 4, 6]. We not infrequently noticed that the subjects took isolated deep breaths, and these were in some cases followed not only by vasostriction, but also, as in Figure 3, by subsequent finger movement.

Simultaneous registration of finger movements and of respiration thus makes it possible to achieve a more objective evaluation of plethysmograph curves.

An insufficiently objective evaluation of plethysmographic data is apparent in the work of a number of authors [7, 8, 11], who have reproduced tracings suffering from obvious distortion due to movements. Thus, for example, Figure 8 of L. B. Perelman's paper [8] shows the vascular reaction recorded from the right arm in response to graded needle-pricks of the left arm. The steep and stepwise decline in the plethysmograph tracings bears a general resemblance to that of Figure 3.

The reaction described by Perelman was observed in a patient in response to a prick, after a series of unfulfilled warnings that the prick was about to take place; this should, in the opinion of the author, have led to an

inhibited state of the cerebral cortex of the subject. In this case the dip in the plethysmograph curve was $2^1/_2$ times greater than before elaboration of the extinguishing inhibition. The author draws the theoretically important conclusion that cortical inhibition causes positive subcortical induction, which affected the nature of the response to the needle-prick.

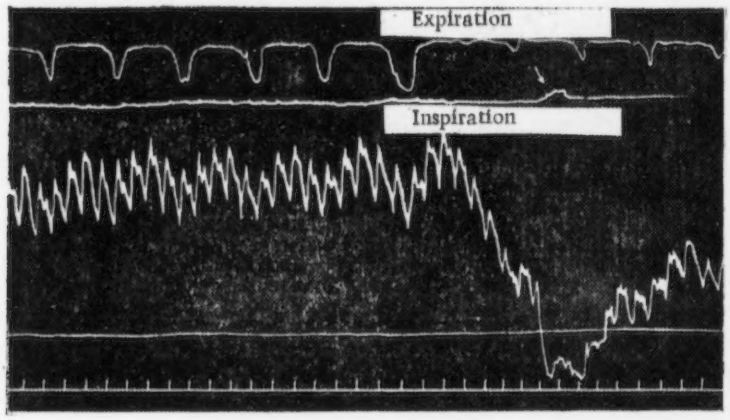


Fig. 3. A single, deep inspiration, constriction of vessels, and movement of the finger, in succession. Explanation of tracings as for Figure 2. The respiratory movements are recorded in the opposite direction to those of Figure 2.

A different conclusion might, however, be drawn if we take into account the almost certain distortion of the recording of the reaction by movements of the patient. During the gradual development of extinguishing inhibition in the cortex an enhancement in excitability may have arisen in some of its regions; for example, in the motor analysor area. A prick inflicted at this stage on the right arm may also have led to a muscle contraction in the other arm, and so have produced the impression that a very strong vasomotor reaction had occurred. It is thus evident that, unless precautions are taken to register movements of the extremity from which plethysmographic recordings are being taken, the value of the data given by plethysmograph of the human is greatly diminished, irrespective of the nature of the stimulus applied.

LITERATURE CITED

[1] V. E. Busygin, Byull. Eksptl. Biol. i Med., 39, No. 6, 71 (1955).

[2] A. P. Kryuchkova, Byull. Eksptl. Biol. i Med., 22, No. 2, 18 (1946).

[3] Kh. S. Koshtoyants, Fiziol. Zhur. SSSR, 21, Nos. 5-6, 1055 (1936).

[4] L. A. Orbeli, Lectures on Problems of Higher Nervous Activity,* 193, 1945.

[5] L. B. Perelman, Zhur. Nevropatol. i Psikhiatr., 52, No. 9, 11 (1952).

[6] M. V. Sergievsky, The Respiratory Center in Mammals, 1950.

[7] Z. N. Serebryakova, Zhur. Vysshei Nerv. Deyatel., 4, No. 3, 348 (1954).

[8] N. I. Strelkova, Voprosy Kurortol., Fizioter., i Lech. Fizich. Kult., No. 3, 15 (1955).

[9] V. N. Chernigovsky and O. S. Merkulova, Byull. Eksptl. Biol. i Med., 22, No. 3, 24 (1946).

[10] V. V. Yakovlev, Byull. Eksptl. Biol. i Med., 15, No. 8, 69 (1955).

[11] A. D. Yarushevich, Vestnik Khirurg. im Grekova 75, No. 3, 80 (1955).

[12] O. Bruns, Munch. med. Wschr., 1924, Bd. 43, S. 3.

* In Russian.

SOME REMARKS RELATING TO THE TECHNIQUE OF INTERPRETATION OF PLETHYSMOGRAPHIC DATA

L. G. Okhnyanskaya

From the Clinical Physiology Laboratory (Director: the late Prof. N. P. Kazunov) Institute of Industrial Hygiene and Occupational Diseases (Director: Prof. A. A. Letavet, Member Acad. Med. Sci. USSR), Acad. Med. Sci. USSR, Moscow

(Received January 14, 1956. Presented by V. N. Chernigovsky, Member Acad. Med. Sci. USSR)

In this paper we present some remarks, of a methodolgoical nature, on the interpretation of plethysmographic data, recorded on the simplest and most widely used model, the Mosso-Novitsky plethysmograph. A number of the remarks may also be applicable to other types of the instrument.

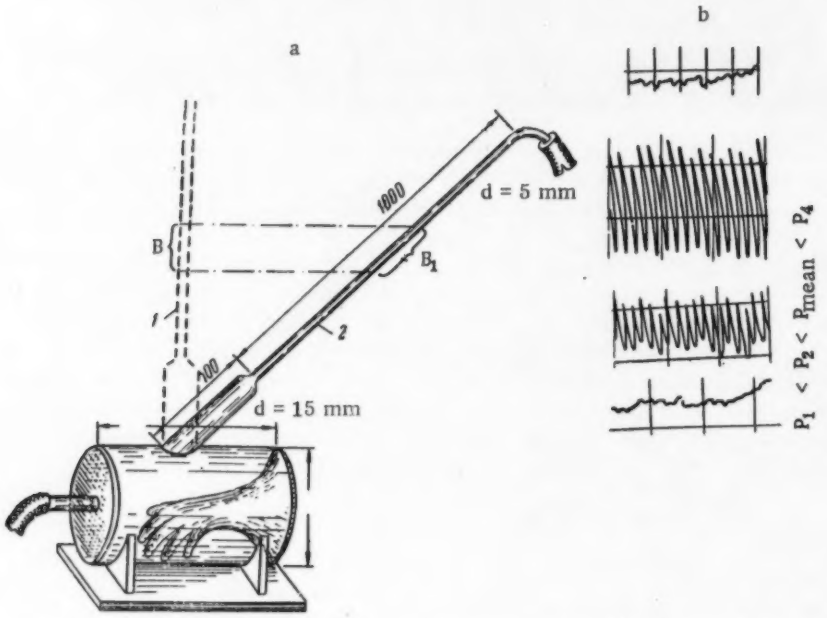


Fig. 1. Schematic representation of a plethysmograph (a), and the amplitude of oscillation at different heights of the column of fluid in the manometric tube of the plethysmograph (b). The ordinates and abscissae are in 1 cm divisions.

Many authors, using the plethysmograph with a constant level of fluid in Tube 1 (Figure 1), find different values for the amplitude of the pulse oscillations in different subjects. Some authors consider the amplitude of the oscillations to be one of the quantitative indicators of vascular tonus. All other factors being equal, the magnitude of these oscillations is a function of the ratio between the external pressure exerted by the column of water in the tube and the internal pressure of the blood in the vessels of the extremity. It will be greatest when the external pressure approaches the average arterial pressure, as is shown by the tracings of Fibure 1, b, in which (from below up) $P_1 < P_2 <$ average $P > P_3$. Following from this, it would seem to us to be more rational to use the instrument at a constant physiological, rather than physical, level, choosing the pressure at which the pulse oscillations are maximum for a given individual, using any other type of plethysmograph. The instrument is under such conditions more sensitive to variations in the volume of the extremity, if the vertical Tube 1 is replaced by Tube 2, inclined at an angle of 30-40° (see Figure 1). Apart from this, with correspondingly equal

changes in the volume of an extremity, the latter is less subject to the effect of the external pressure exerted by the column of water.

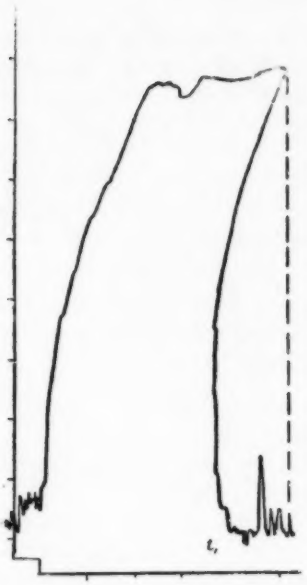


Fig. 2. Movements of the stylus during development of a direct and a return reaction.

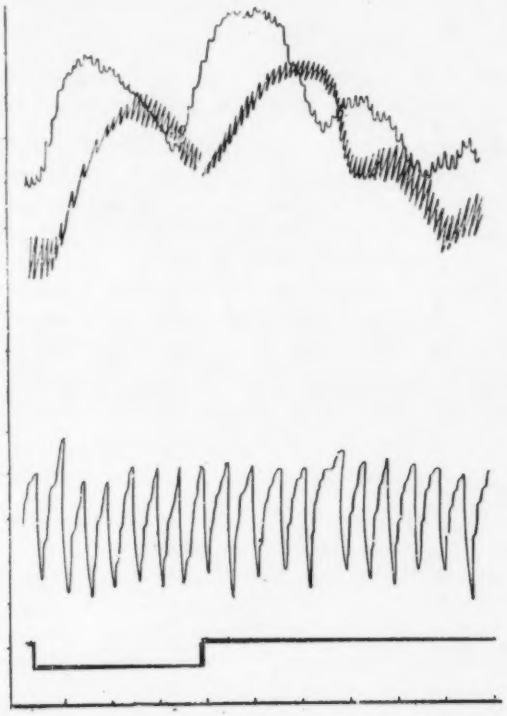


Fig. 3. Example of spurious asymmetry, in the reaction to counting from 20 to 1. Explanation of curves (from above down): plethysmogram, from the left hand, from the right hand, pneumogram, signal showing time and duration of action of the conditioned and unconditional stimuli (the former act over the whole duration of the latter), time signal (5 second intervals). Upward deviations from the initial level indicate, respectively: on the plethysmogram, diminution in volume of the extremity; on the pneumogram, expiration; downward deviations similarly indicate: on the plethysmogram, increase in volume of the extremity; on the pneumogram, inspiration. The graduations of the ordinate axis are each 1 cm, and of the abscissa, 5 second intervals.

In examining a plethysmogram, we pay particular attention to the height of the waves, to their velocity of development, and to the symmetry or lack of symmetry of the reactions. However, plethysmograms do not always give a correct picture of the changes in volume of the extremity concerned. Comparisons of the magnitudes of different reactions are usually based on the amplitude of the fluctuations in volume, without taking into account that the relation between the displacement of the stylus and that of the water in the plethysmograph tube is not a linear one. It is for this reason necessary to calibrate the instrument so as to determine the dependence (expressed graphically) between the displacement of the water level in the plethysmograph tube and the displacement of the stylus. The comparative evaluation of the magnitude of a reaction should be based not on the amplitude of the oscillations seen in the tracings, but on the corresponding changes in the level of the water column in the plethysmograph Tube 2, as derived from the calibration curve. Calibration gives one the possibility of comparing data obtained from different instruments.

The above remarks on the magnitude of the reaction also apply to the speed of development of the direct and return reactions. A precise assessment of the speed of development of a reaction is complicated by the circumstance that the vertical movements of the stylus are along an arc, and not along a straight line (Figure 2). For this reason, the frequently applied method of assessing time as being the point of projection of a curve on the abscissa axis gives an incorrect value; for example, in Figure 2 the return reaction is completed not at

time t_1, but at a slightly greater time t_2, the exact evaluation of which requires a knowledge of the rate of displacement of the stylus and of the radius of the arc described by it.

It is evident from the foregoing remarks that the generally accepted procedures for the evaluation of the magnitude and speed of the reactions are not accurate, and require the introduction of corrections.

Since it is not possible to achieve identical tensions of the rubber of the Marey tambours, then, even if the styluses are of identical length and weight (which is in practice highly improbable), the displacements registered on the tracings will be different, although the volume changes in the two extremities may have identical. This gives rise to an apparent difference in the rates of development of the reaction in the two extremities, and to a spurious asymmetry of reaction, applying not only to its magnitude and speed of development but also to its direction at a given moment (Figure 3). This effect is due to one stylus lagging behind the other. It is, in order to establish asymmetry in magnitude and speed of a reaction, essential to switch the recording system over from the left to the right hand, or vice versa. Only then can we speak with assurance of the existence of asymmetry. Asymmetry in the direction of the reactions can be regarded as real only if we are able, visually or otherwise, actually to observe at any moment that the styluses are moving in opposite directions.

The routine registration of plethysmograms from both hands may serve as a reliable method of detecting distortions due to imperfections in the equipment.

In some cases we find that the plethysmograms take a very long time to return to their initial levels, both of the height of the tracing and of the amplitude of the oscillations. Such deviations may be the results of physiological changes connected with the prolonged alteration in the volume of the extremity. Special experiments showed, however, that changes in the volume of the part of the body within the plethysmograph are reflected not only in changes in tension in the receiving tambour, but also partly in the position of the wall of the plethysmograph which is a part of the rubber glove, lined with cotton wool, and bound to the apparatus. Such a wall cannot be considered to be "rigid," and it may often give rise to prolongation of the return of the plethysmogram to its initial level. We apply the following technique to the detection of this possibility. The whole system is connected with the atmosphere. If after this the gradual fall in the level of the tracing continues, the effect is a physiological one, while if it remains more or less constant the effect is of physical origin.

It follows from the above considerations that it is necessary to continue work on the designing of more reliable, although simple, instruments, of a sensitivity adequate for the given purpose, and within the means of a wide range of users.

RECORDING OF ELECTROGRAMS OF WORKMEN DURING INDUSTRIAL WORK

L. A. Vodolazsky

From the Laboratory of Physiology of Labor (Director: Prof. S. A. Kosilov, Doctor Biol. Sci.), Institute of Industrial Hygiene and Occupational Diseases, Acad. Med. Sci. USSR (Director: Prof. A. A. Letavet, Member Acad. Med. Sci. USSR)

(Received November 3, 1955. Presented by V. N. Chernigovsky, Member Acad. Med. Sci. USSR)

At present, physiologists studying the physiology of labor register electromyograms (EMG) and electroencephalograms (EEG) of subjects during performance of work under laboratory conditions in screened rooms, whereas electrocardiograms are recorded in the factory itself, using standard techniques, as close as possible to the workplace of the subject.

We undertook the problem of registering ECG and EMG recordings of persons working in industry, simultaneously with recordings of their industrial activities.

Having become acquainted with the techniques of registering bioelectrical activities in the clinic, and of taking the ECG and other bioelectrical records from aircraft pilots in flight (applied in aviation medicine),

involving radio transmission of electrophysiological information from the subject, and having investigated the limitations of electrography and the possible artifacts which might arise in metal working shops, we arrived at the conclusion that it is possible to take satisfactory ECG and EMG recordings from workmen performing their work in the factory itself, under ordinary bench conditions.

Since the movements of the workmen give rise to artifacts of muscular origin, which distort the ECG taken using standard leads, we applied the known procedure of placing the electrodes at such points of the chest of the subjects as would ensure maximum amplitude of the ECG, with minimum muscle potentials. Thus in the tracings of Figures 1 and 2 the ECG was recorded with electrodes in the pectoral position at CR_4 and on the back

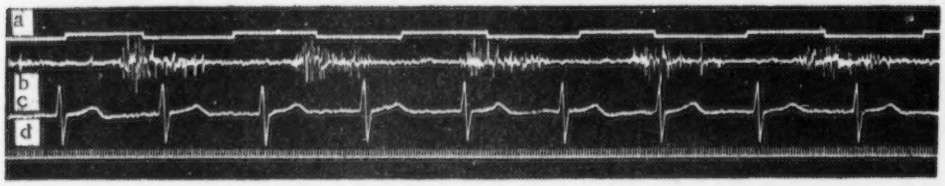


Fig. 1. Electrogram recorded during cutting of metal with a chisel. a) recording of impact of the hammer with the chisel (downward displacement of the line); b) EMG taken from the triceps muscle of the right arm; c) ECG; d) time signals (50 seconds).

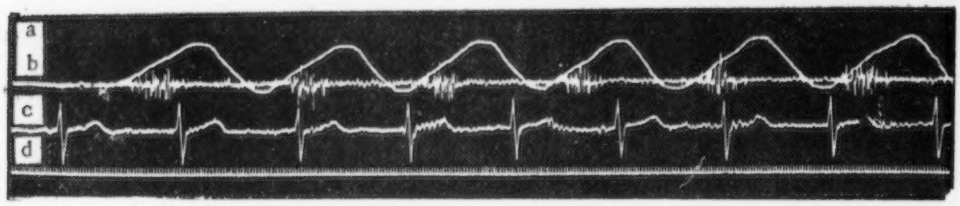


Fig. 2. Electrogram recorded during filing of metal. a) recording of movements of the file in a horizontal plane; b), c), and d) as in Figure 1.

between the rectus and oblique muscles. This arrangement of the electrodes permitted of satisfactory registration of ECG during cutting or filing of metals. It is also possible to have the electrodes at other locations.

Alterations in resistance in the area of contact of the electrodes with the skin give rise to variable emf effects, which cause distortions of the electrograms. For this reason we paid special attention to the attachment of the electrodes to the skin, and to the electrode paste used. We used cup electrodes, stuck to the skin with "Kleol," and VNIIMIO electrocardiograph electrode paste.* The magnitude of the resistance between the electrodes is also of considerable importance: if it is great, the possibility of leading in alternating current increases. We used electrodes of 15 mm diameter for the ECG, and of 8 mm diameter for the EMG. With these, the resistance between the electrodes was 1-3,000 ohms for the ECG recordings, and 10-15,000 ohms for the EMG, and these values were achieved by thorough preparation of the skin before applying the electrodes.

In order to eliminate E.M.F. interferences arising during displacements of the leads we covered them with flexible shielding material, almost to the electrodes, and we connected the shielding material with the casing of the amplifier. This shielding was quite adequate for recording of ECG and EMG during voluntary movements of the subjects.

* VNIIMIO Paste No. 3, for use with lead electrodes, has the composition: starch 7%, stannous chloride 7%, finely powdered pumice 26%, glycerol 7%, "Diotsid" 0.05%, distilled water 53%.

It should be borne in mind that, in recording EMG of subjects making violent movements in a screened room, it is also necessary to use shielded electrode leads.

The chief shource of interference with electrography encountered in metal working shops is to be found in the variable electrical field of the power and lighting systems.

In order to eliminate these sources of interference we used a differential amplifier, connecting the body of the subject to its housing by means of a "null" electrode. With the resistances between the electrodes as mentioned above, the amplifier should have a discrimination coefficient of only about 1,000-1,500.

The equipment used by us for ECG and EMG recording at a workshop bench consisted of a two-channel amplifier and an oscillograph. Devices transforming elements of the movements of the workmen into electrical impulses were connected to the oscillograph. The input points of the amplifier were connected through shielded leads to a plug fastened to a belt worn by the workmen. Single shielded leads connected the plug to the electrodes.

The equipment had an independent current supply. In many cases the equipment did not need to be grounded.

We made ECG and EMG recordings, using the above-described equipment, from persons working in a metal-working shop. Figure 1 shows the electrogram recorded during cutting of metal with a chisel, and Figure 2 that during filing of metal. In addition, we took ECG and EMG recordings of men engaged in drilling metal on a lathe and with an electric drill, and in working a shaping lathe.

In working with grounded equipment it should be borne in mind that the subject is also grounded and strict safety precautions should be observed.

Should it be necessary to record a one-channel ECG, this may be done using the EKP-4 electrocardiograph, or some similar instrument. In such cases the electrode leads should be shielded.

Our results show that electrographic methods may be applied to the study of bioelectric processes taking place during work in a factory, and this facilitates the solution of a number of problems of the physiology of work under industrial conditions.

INSTRUMENT FOR NARROWING THE LUMEN OF THE AORTA, IN SHORT EXPERIMENTS

A. Kh. Khaidarov

From the Laboratory of Clinical Physiology (Director: Prof. P. K. Anokhin, Member Acad. Med. Sci. USSR), A. V. Vishnevsky Institute of Surgery, Acad. Med. Sci. USSR (Director: Prof. A. A. Vishnevsky, Corresponding Member Acad. Med. Sci. USSR)

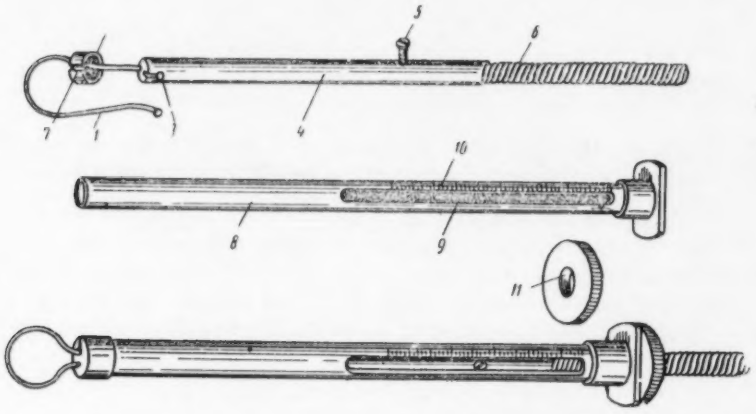
(Received May 18, 1956. Presented by P. K. Anokhin, Member Acad. Med. Sci. USSR)

A study of arterial pressure levels, and of depressor nerve activity, in experimental aortic stenosis is of considerable importance to the development of modern cardiovascular surgery.

The existing instruments for determining the degree of narrowing of the lumen of the aorta are very imperfect [1, 2]. We were not able, from a search of the literature available to us, to find a single description of an instrument which would be simple in operation, and which would produce accurately any required degree of stenosis of the aorta, under conditions of short experiments in the laboratory.

The instrument devised by us (see Figure) consists of a metal cylinder, containing a threaded piston rod, a nut, a cap, and a movable guide. The cylinder is 12.5 × 0.5 cm. A window is cut in the upper third of its length, dimensions 5 × 0.5 cm, to enable movements of the piston rod to be observed. A scale is engraved on the right-hand side of the window, graduated in mm from 0 to 20 mm. The rod consists of three portions: a thread is cut

into the upper portion, serving for moving the rod along the cylinder, a pointer is inserted into the middle third, serving for indicating the degree of stenosis achieved, and a hole, dimensions 3 × 3 mm, is drilled through the lower end of the rod, with a notch at each opening leading to the end of the rod. The ends of a loop are fitted into the hole and notches. Slits are cut on each side of the cap, and an opening is made in the top, through which the loop passes. The loop is made of tough, flexible material, and has a diameter of 20 mm.



Instrument for narrowing the lumen of the aorta. 1) oop; 2) cap; 3) opening and notches in the piston rod; 4) piston rod; 5) pointer; 6) threaded end of piston rod; 7) slit in cap; 8) cylinder; 9) window in cylinder; 10) scale; 11) nut.

Method of use of the instrument. The animal is anesthetized, and the carotid and iliac arteries are connected to a mercury manometer. Depressor nerve impulses are registered with the aid of an oscillographic equipment. The normal blood pressure and activity of the depressor nerve are recorded before stenosing the aorta. A transverse incision is then made on the left side, at the level of the 5th-6th ribs, and the thoracic cavity is opened layer by layer. One end of the loop is passed under the descending arc of the aorta and fitted into the notch at the end of the rod, so that the aorta is fully encompassed by the loop, which assumes the shape of its cross-section. The diameter of the aorta is then read from the scale, and the knurled nut is turned, so as gradually to narrow the lumen of the aorta until a rise in carotid artery pressure, and a fall in iliac artery pressure, are noted, together with alteration of impulsation from the depressor nerve.

The narrowing of the lumen of the aorta is then read from the scale, in mm. Knowing the diameter of the aorta before and after constriction we calculate the area of the cross-section of the aorta from the formula $S = \pi R^2$. Having determined these cross-sections, we can readily calculate the percentage narrowing of the lumen.

The instrument was used experimentally on 15 dogs and 25 rabbits. It enabled us to grade the degree of stenosis of the aorta, and to operate with known aortic diameters throughout an experiment; using the appropriate equipment, it made it possible to relate the arterial pressure and the activity of the depressor nerve to the degree of aortic stenosis.

LITERATURE CITED

[1] F. Z. Meerson and G. V. Kobozev, Byull. Eksptl. Biol. i Med., 39, No. 5, 50-52 (1955).

[2] R. G. Ellison, R. C. Major, R. Pickering, W. Hamilton and J. Thor, Surgery, 1952, Vol. 24, p. 2.

[3] J. H. Gibbon, M. Hopkinson and E. D. Churchill, J. Clin. Invest., 1932, Vol. 11, pp. 543-553.

MEASUREMENT OF HEIGHT OF ELECTROCARDIOGRAM WAVES

D. P. Kochetov and B. S. Tikhonov

From the Chair of Venereal and Skin Diseases, S. M. Kirov Gorky State Medical Institute, and the Gorky Scientific Research Dermatological-Venereological Institute (Director: Prof. M. P. Batunin)

(Received August 20, 1955. Presented by Prof. V. N. Chernigovsky, Member Acad. Med. Sci. USSR)

In recent times, not only the form of the waves of electrocardiogram (ECG) tracings, but also their height, i.e., the voltage, are considered in reading diagnostic or experimental ECG records.

The height of the ECG waves is usually measured in mm, and it also shows the scale of the recording, and the value of a millivolt on the scale (the size of a millivolt expressed in linear units (mm) of the height of a wave).

It is often very difficult to compare the dimensions of the waves, i.e., their voltage, of electrocardiograms taken at different times and at different amplifications, i.e., with different scales of millivolt dimensions. It is then necessary to express the wave dimensions in millivolts, which involves the laborious measurement of wave heights, followed by conversion of linear units to millivolts; all this is most time-consuming, and is for this reason seldom carried out in practice.

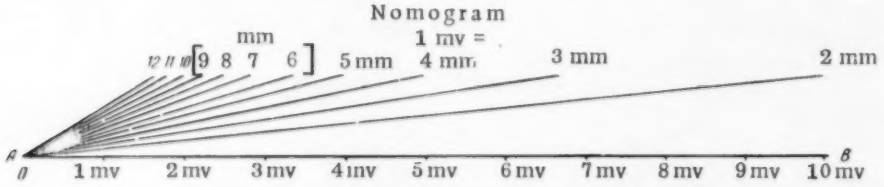


Fig. 1.

Fig. 2.

We here present a table and a nomogram, designed to facilitate and expedite the conversion operation. The Table gives the most commonly encountered dimensions of ECG waves (from 0.5 to 20 mm), expressed as millivolts at different scales of recording, i.e., with equivalent heights of a millivolt of from 2 to 12 mm.

The first vertical column of the Table gives the scale, i.e., the magnitude of a millivolt expressed in mm, while the top horizontal row gives the height of the ECG waves in mm. The height of the waves in millivolts are given by the figures at the intersection of the appropriate horizontal rows and vertical columns.

Thus, for example, the height of an R wave of an ECG is found, using one of the leads, to be 11 mm with a millivolt scale of 7 mm. The value given for 11 mm in the vertical column and 7 mm in the horizontal row is 1.57 millivolts, which is the potential of the given wave.

A nomogram is shown in Figure 1. The base AB of the nomogram gives the linear representations of the height of the ECG waves in millivolts (mv), the scale chosen being 200 mm per 10 mv, whence it follows that 20 mm corresponds with 1 mv, and 1 mm with 0.05 mv. According to the precision required, the scale may be enlarged or made smaller.

TABLE

Table for Conversion into Millivolts of the Height in mm of ECG Waves.

Millivolt scale (mm)	Height of wave in mm																							
	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0	13.0	14.0	15.0	16.0	17.0	18.0	19.0	20.0
2	0.25	0.5	0.75	1.00	1.25	1.50	1.75	2.00	2.25	2.50	3.00	3.50	4.00	4.50	5.00	5.50	6.00	6.50	7.00	7.50	8.00	8.50	9.00	9.50	10.00
3	0.17	0.33	0.50	0.67	0.83	1.00	1.17	1.33	1.50	1.67	2.00	2.33	2.66	3.00	3.33	3.67	4.00	4.33	4.67	5.00	5.33	5.67	6.00	6.33	6.67
4	0.12	0.25	0.37	0.50	0.62	0.75	0.87	1.00	1.12	1.25	1.50	1.75	2.00	2.25	2.50	2.75	3.00	3.25	3.50	3.75	4.00	4.25	4.50	4.75	5.00
5	0.10	0.20	0.30	0.40	0.50	0.60	0.70	0.80	0.90	1.00	1.20	1.40	1.60	1.80	2.00	2.20	2.40	2.60	2.80	3.00	3.20	3.40	3.60	3.80	4.00
6	0.08	0.17	0.25	0.33	0.41	0.50	0.58	0.67	0.75	0.83	1.00	1.16	1.33	1.50	1.67	1.83	2.00	2.17	2.33	2.50	2.67	2.83	3.00	3.17	3.33
7	0.07	0.17	0.21	0.28	0.36	0.43	0.50	0.57	0.64	0.71	0.85	1.00	1.14	1.28	1.43	1.57	1.71	1.86	2.00	2.14	2.28	2.43	2.57	2.71	2.86
8	0.06	0.12	0.19	0.25	0.31	0.37	0.43	0.50	0.56	0.62	0.75	0.88	1.00	1.12	1.25	1.37	1.50	1.62	1.75	1.87	2.00	2.12	2.25	2.37	2.50
9	0.05	0.11	0.17	0.22	0.28	0.33	0.39	0.44	0.50	0.56	0.67	0.78	0.89	1.00	1.11	1.22	1.33	1.44	1.55	1.67	1.78	1.89	2.00	2.11	2.22
10	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50	0.60	0.70	0.80	0.90	1.00	1.10	1.20	1.30	1.40	1.50	1.60	1.70	1.80	1.90	2.00
11	0.04	0.09	0.14	0.18	0.23	0.27	0.31	0.36	0.41	0.45	0.54	0.64	0.73	0.82	0.91	1.00	1.09	1.18	1.27	1.36	1.45	1.54	1.64	1.73	1.82
12	0.04	0.08	0.12	0.17	0.21	0.25	0.29	0.33	0.37	0.41	0.50	0.58	0.67	0.75	0.83	0.91	1.00	1.08	1.17	1.25	1.33	1.42	1.50	1.58	1.67

At point A of Figure 1 we draw a number of lines at different angles to the base-line AB of the nomogram, each for a different linear magnitude of a millivolt, from 2 to 12 mm.

To know the potential of an ECG wave we must first know the equivalent height of a millivolt, in mm (i.e., the scale of a millivolt). The linear height of a wave, measured with the aid of a pair of dividers, is marked on one of the sloping lines of the same millivolt equivalent, and a perpendicular is drawn from this point to the base-line; the point of intersection will give the required result. For example, with an equivalent height of a millivolt of 11 mm (Figure 2), the linear height of a wave (distance between the legs of a pair of dividers) is equal in length to a perpendicular erected on the line AB at a distance of 16 mm from A. On the given scale, the potential of the given wave will be 0.8 mv.

Geometrical basis of the nomogram

The nomogram is constructed on the principle of similar triangles. Taking a maximum ECG wave height of 20 mm we determine its value in millivolts, for a millivolt equivalent of 2 mm, 3 mm, etc., up to 12 mm, either experimentally or from the Table, and the resulting values are marked off on the base-line AB of the nomogram. From these points we erect perpendiculars 20 mm in height, and join the tops of these perpendiculars to point A. For example, a wave 20 mm high, at a millivolt scale of 11 mm, corresponds with a value of 1.82 mv. At the given scale, this value will be represented on AB by the point D, at a distance of 36 mm from A (see Figure 2). From this point we erect a perpendicular DC, equal to the maximum height of the wave, viz., 20 mm. The top of this perpendicular is joined to A.

The heights of all the perpendiculars to the base AB (wave heights) of the triangle formed are proportional to the corresponding lengths of the bases of the triangles, since the triangles so constructed are similar ones. If the perpendicular DC (wave height in mm) corresponds to the section AD (expression of wave potential in mv), then all the sections perpendicular to AD will correspond with sections from point A to the point of intersection of the perpendiculars with AD, i.e., with their value in mv.

It is in practice more convenient to draw the nomogram on sheet metal or celluloid, with a movable ruler fixed at point A, connecting it with any desired millivolt equivalent scale point, which saves the trouble of drawing the sloping lines for each millivolt equivalent scale.

NEW MODEL OF EXPERIMENTAL HYPERTENSION PRODUCED BY ISCHEMIZATION OF THE SPLEEN

F. A. Morokhov

From the Chair of Pathological Physiology (Acting Director: Assistant Prof. F Morokhov) Yaroslavl Medical Institute (Scientific Director: Prof. A. M. Dubinsky)

(Received October 1, 1955. Presented by V. N. Chernigovsky, Member Acad. Med. Sci. USSR)

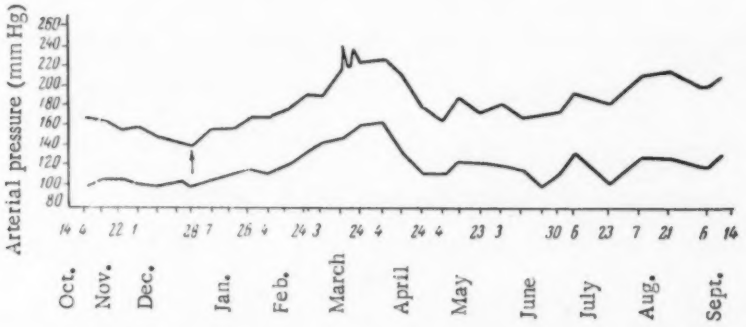
Experimentally induced hypertensive states serve as models for the study of the disturbances associated with this condition, from which conclusions may be drawn respecting the initiation and development of those physiological processes present in the diseased organism which lead to the prolonged elevation of blood pressure, so providing an approach to the elucidation of the pathogenesis of hypertensive disease. The variety of methods which have been applied for the elicitation of experimental hypertension is indicative of the common pathogenesis of the hypertensive states produced. In our opinion, this general factor is characterized by the initiation of trophic and vasopressor reflexes and by changes in metabolism and production of vasopressor substances. As has been shown by numerous researches, the latter are formed as a result of hypoxia of ischemic organs, with disturbances of their trophism.

As far back as 1870 I. O. Navalikhin, and in our times N. N. Anichkov [1] and others have shown that experimental ischemia of the brain is followed by a rise in blood pressure. Experimental hypertension following ischemization of the kidneys was shown to occur by Ya. Ya. Stolnikov [5], and later by Goldblatt [3] and numerous others. More recently fairly prolonged hypertensive states have been found to result from cardiac and uterine ischemia [2, 4]. It is not yet known, however, whether ischemia of other organs, in particular the spleen, plays any part in the pathogenesis of hypertensive disease.

The present paper deals with the production of experimental hypertension by causing ischemia of the spleen (G. V. Kasatkina took part in the experiments).

EXPERIMENTAL METHODS AND RESULTS

Our experiments were conducted on 15 dogs of different sexes and ages. One of the carotid arteries was displaced to a subcutaneous position or to a skin pedicle. The dogs were then familiarized with laboratory conditions and with those of the experiment, over a period of two months. Blood pressure was measured daily for each dog in a special room, by Korotkov's method, and the pulse and respiratory rates were recorded. After the dogs had become accustomed to this routine they were subjected to laparotomy. The abdomen was opened, under morphine-ether anesthesia, and under aseptic conditions, and a number of branches of the splenic vein and artery were ligated, after which the spleen was inserted into a subcutaneous pouch formed in the abdominal wall. In other dogs we constricted the splenic artery and one or two arterial branches, shifted the spleen to the abdominal cavity, and closed the wound.



Alterations in blood pressure observed in the dog Jack after producing splenic ischemia. Explanation of tracings (from above down): maximum arterial pressure, minimum arterial pressure, ↑ when operated.

The dogs all withstood the operation well. They showed no noticeable subsequent behavior changes. Arterial pressure began to rise gradually in all the dogs soon after the operation, from initial levels of 138-140/99-100 mm Hg to 241/100 mm Hg $1^1/_2$-2 months later, and in some cases to even higher levels. After this, arterial pressure gradually declined, probably as a result of compensation of the experimentally produced disturbances in the circulation and metabolism of the spleen.

Alterations were also observed in the pulse and respiration: the pulse rate rose by 30-40 beats per minute, and the respiratory rate by 10-12 respirations per minute.

The carotid arteries of the dog Jack were fixed in a subcutaneous position. During $2^1/_2$ months of control observation of the general condition of the animal and of its blood pressure, pulse, and respiration, we proceeded to the operation. Before the operation arterial pressure was 142/99 mm Hg, pulse rate 86, and respiration 19 per minute. We ligated two branches of the splenic artery, and shifted the spleen to the peritoneal cavity. The wound was then closed, and it healed uneventfully. As appears from the Figure, arterial pressure rose gradually after the operation, to reach a level of 243/145 mm $2^1/_2$ months later. After two weeks it began gradually to fall, but did not reach the initial level over the period of observation. It is probable that complete return to normal pressure was prevented by a skin disease which supervened (hyperemic patches on the head and body, with falling out of hair and formation of boils). The affected areas of skin were painful. Similar skin conditions were seen in three other dogs. These dogs, similarly to Jack, showed a rise in blood pressure; ischemization of the spleen had not been carried out for one of them. Together with rise in arterial pressure, we observed acceleration of the pulse, from 86 before the operation to 118 on the 3rd day after it, and to 130 after 3 months. Rise in respiratory rate was less marked.

A different variant of the operation was applied in the case of the dog Bobik. After laparotomy, two branches of the splenic artery and one splenic vein were ligated. The spleen was moved to a skin pouch in the wall of the abdomen, and the wound was closed in layers. After a number of days arterial pressure began slowly to rise, reaching a maximum of 194/132 mm after 2 months, i.e., rather sooner than for the dog Jack, and then began to fall. During the first few days after the operation the pulse rate rose from 93 to 118 beats per minute. The respiratory rate increased from 20 preoperational to 28 after the operation.

The respiratory rate reverted to the initial frequency sooner than the pulse. Development of hypertension proceeded in the same way in the other animals as for the ones described above.

DISCUSSION OF RESULTS

We think we are justified in remarking that one is not justified, in the present state of knowledge, in limiting secondary pathogenic factors of hypertensive disease only to renal ischemia. The published data, as well as our findings that experimental hypertension may be produced as a result of ischemia of the spleen, are evidence of the role played by other viscera, liable to ischemia and to anoxic metabolism, in the pathogenesis of the disease. We have no information regarding the relative importance of splenic ischemia, as compared to that of other organs, in the pathogenesis of hypertensive disease. This question, and also that of the elucidation of the actual physiological mechanism of development of hypertension in our experiments will be the subjects of our further research.

LITERATURE CITED

[1] N. N. Anichkov, Vestnik Khir. Pogran. Obl., Vols. 16-17, Nos. 48-49, pp. 105-109 (1929).

[2] M. A. Volin, E. S. Tsvilikhovskaya, T. I. Beslekoev, and V. S. Mayat. Terap. Arkh., 22, No. 6, 17-25 (1950).

[3] Goldblatt, cited from Klin. Med., 17, No. 8, 4-9 (1939).

[4] P. D. Gorizontov and D. S. Shlyapin, Arkhiv Patol., No. 3, 13-17 (1948).

[5] Ya. Ya. Stol'nikov, Archives of Clinical Internal Diseases* by S. P. Botkin, Vol. 6, pp. 306-308 (1880).

* In Russian.

TECHNIQUE OF EXPERIMENTAL PRODUCTION OF A VENOUS THROMBUS

Z. D. Bleksmit and M. A. Kotovshchikova

From the Dry Preparations Laboratory, Leningrad Blood Transfusion Institute (Director: Prof. L. G. Bogomolova)

(Received December 8, 1955. Presented by V. N. Shamov, Member Acad. Med. Sci. USSR)

Methods have been reported in Russian and foreign publications, for the production of thrombi in living animals by chemical or mechanical agents, or using thromboplastin, staphylococcus toxin, ferric chloride, hypertonic sucrose solutions, and other substances.

These methods do not, however, fulfill their purpose of producing a thrombus in a live animal.

We have made use of the technique proposed by Hirsch and Lowe [1] with slight modifications.

The animal (a rabbit) was fastened to a board in a supine position. The skin was incised in the mid-cervical line. A 3-4 cm length of the jugular vein was dissected free. The proximal and distal ends of the vein were caught up in ligatures, so as to facilitate lifting it. The ligatures were not tightened. The flat handle of any instrument was inserted under the section of vein, and the vein was given 20-35 light taps with the flat of a scalpel handle. After 2-4 minutes the vein was seen to blanch, and a formed thrombus could be felt within it. The ligatures were then removed. Slight bleeding was readily stopped by pressure with a gauze plug.

EXPERIMENTAL RESULTS

We were able to produce thrombi in 15 veins (one in the iliac vein) of 8 rabbits. Histological examination, conductred under the direction of V. P. Teodorovich, confirmed the presence of a fresh thrombus in every case. It is obvious that the given method introduces all the factors needed for the formation of a thrombus: temporary stoppage of blood flow, injury of the intima, and release of thrombokinase.

The above-described method is simple and affords a convenient means of producing a model of thrombosis in rabbits, for the experimental study of anticoagulants.

LITERATURE CITED

[1] E. Hirsch and L. Lower, Proc. Soc. Exp. Biol. a. Med., 1946, Vol. 63, No. 3 pp. 569-572.

EFFECT OF ABLATION OF THE CEREBRAL CORTEX ON THE SECRETORY ACTIVITY OF THE SALIVARY GLANDS OF DOGS

T. A. Maltseva

From the Chair of Normal Physiology (Director: Prof. E. A. Asratyan, Corresponding Member Acad. Med. Sci. USSR), I. V. Stalin II Moscow Medical Institute (Director: Assistant Prof. S. I. Milovidov)

(Received January 2, 1956. Presented by P. S. Kupalov, Member Acad. Med. Sci. USSR)

The first researches on the cortical control of salivation were reported by Lepine and Bochefontaine [15], over the years 1875-1888. V. M. Bekhterev and N. A. Mislavsky [2] found, in short experiments on dogs, that direct stimulation of certain cortical areas caused salivation on the side stimulated. G. P. Zelenyi, L. A. Klemenkova, and D. S. Fursikov [9, 10] observed a diminished flow of saliva in totally decerebrate dogs during

meat feeding or when acid solutions were placed in the mouth. D. A. Biryukov [3, 4] found that immediately after removal of one or "one and a half" hemispheres of dogs there was a certain increase in the unconditioned reflex secretion of saliva, but that with time the preoperational rate of secretion returned. Ya.M. Pressman [14] found that unconditioned reflex discrimination between various stimuli was coarser in decerebrate dogs than in normal ones.

The influence of the cerebral cortex on unconditioned reflex activity of the salivary glands has not yet, however, been fully elucidated.

EXPERIMENTAL METHODS

The experiments were performed on three dogs: Malchik , Ryzhik, and Tobik. Both parotid glands of the dogs Malchik and Ryzhik were provided with fistulae, while Tobik had one parotid and one submaxillary gland fistula.

Two series of experiments were performed. In the first series we measured the secretion from each gland during a short feed with dry food: 1 g portions of white rusks or of meat powder every 30 seconds for 15 minutes. Over this time, the saliva was collected in graduated cylinders, separately for each gland.

In the second series we followed salivation in response to stimulation of the buccal mucosa with hydrochloric acid of various concentrations (20 ml of 0.1, 0.3, and 0.5% acid per 30 seconds). The acid was introduced into the mouth by means of a special dispenser attached to the inner surface of the cheek, which allowed us to measure the secretion of each gland, depending on the side stimulated. The saliva was collected for 3 minutes after applying the stimulus.

After we had determined the amount of saliva secreted, and ascertained the relative activities of each of the paired glands in normal dogs, Prof. E. A. Asratyan performed the operation of removing the cortex of one cerebral hemisphere. The cortex of the left cerebral hemisphere was removed from Malchik and Ryzhik (21.5 g of brain was taken from Malchik, who weighed 8 kg, and 16. 5 g from Ryzhik, who weighed 6.5 kg). The right cerebral cortex, weighing 23.5 g, was taken from Tobik (weight 10 kg).

After recovery from the operation (13-17 days) the dogs fed themselves, and moved around freely; the experiments were then renewed. The techniques were identical with those applied before the operation.

In all, 78 experiments were performed on Tobik, 68 on Malchik, and 52 on Ryzhik. Tobik and Malchik were kept under observation for 18 months after the operation, and Ryzhik for 6 months.

EXPERIMENTAL RESULTS

An examination of the secretory function of the parotid glands showed that their activities were not uniform under normal conditions. The amount of saliva secreted by each gland varied from experiment to experiment.

Where the flow of saliva was stimulated by acid, the dependence of the amount secreted on which side was stimulated was evident — salivation was always greater on the side at which acid was introduced.

Our results are in accordance with those of other authors who had studied the activities of the parotid glands [1, 4, 5, 6, 14].

The differences between the secretory activities of the two glands are not, however, very great under normal conditions; the ratios of the mean values are 1 : 1 or 1 : 1.06 (Figure 1).

Removal of the cortex of one of the cerebral hemispheres resulted in a considerable alteration in the secretory function of the salivary glands. This alteration was of the same nature in all the dogs.

In the first place, there was a considerable diminution in secretion from the gland on the operated side. Thus, the average secretion of the left parotid gland of the dog Malchik fell by 41.7% after ablation of the left cerebral cortex. That of the right parotid gland of the dog Tobik fell by 34% after removal of the right cerebral cortex (Figure 2).

In the second place, secretion slightly increased on the side opposite to the operated one. Thus the average secretion of the right parotid gland of the dog Malchik rose by 28%.

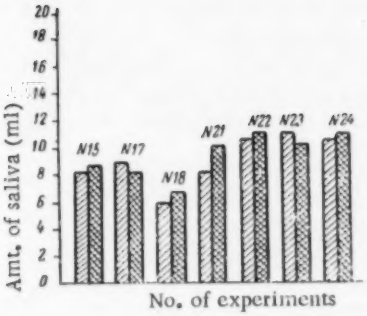


Fig. 1. Flow of saliva, in ml, from the left and the right parotid glands of the dog Ryzhik, under normal conditions.

In the third place, owing to the changes in the relative activities of each of the paired glands, there was a striking change in the ratio of their activities, from 1 : 1 preoperational to 1 : 1.7, 1 : 2, and even 1 : 3.

Apart from this, we noticed that more saliva was secreted by the left gland of normal dogs on some occasions, and by the right gland on others, whereas after operation the flow was always less on the operated side (Figure 3).

In the experiments involving introduction of acid, excess of secretion on the side at which the acid was put in was no longer the rule; the flow of saliva was always less on the operated side.

The changes in the secretory function of the glands appeared soon after the operation (the experiments on Ryzhik were commenced on the 13th, and on Malchik on the 17th day after the operation), and persisted over a year or more. During the second year after the operation a certain increase in the flow of saliva in response to meat powder was observed for the dog the average flow rising from 12 to 15 ml from the right gland, and from 7 to 8.8 ml from the left. The ratio of the secretions remained constant, however.

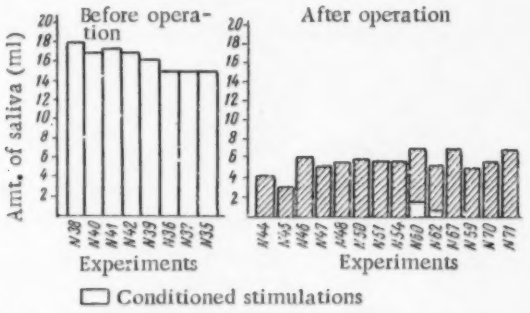


Fig. 2. Flow of saliva, in ml, from the right submaxillary gland of the dog Tobik before and after removal of the right cerebral cortex.

In some of the experiments with operated dogs we investigated the natural conditioned reflexes to the smell and sight of food. We found that the conditioned reflex flow of saliva had altered in the same way as had unconditioned reflex flow, viz., the gland on the operated side secreted much less than on the intact side. Thus over 6 minutes during which Malchik was exposed to the sight and smell of meat powder his left gland (operated side) secreted 0.2 ml of saliva, and his right gland 1 ml. Similarly, Ryzhik's left gland secreted 0.1 ml of saliva, and his right gland 1 ml.

The results of our experiments are in accord with the observations of S. L. Levin [12], who studied unconditioned reflex secretion of saliva in patients suffering from cerebral tumors and brain injuries.

Paired organs are particularly valuable for experiments designed to elucidate the influence of the cerebral cortex on the activity of these organs.

In our experiments one gland served as a control for the other. This was of assitance in enabling us to find the causes for the changes in the functions of the organ after decortication. The impairment of the function of the salivary gland on the operated side might be explained as resulting from the severe trauma inflicted on the central nervous system by the operation. However, the unfavorable effects on the organism of so serious an operation as unilateral decortication would be expected to affect the functional state of both glands equally. Yet

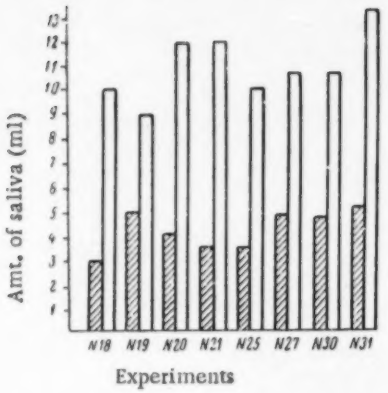


Fig. 3. Flow of saliva, in ml, from the left and right parotid glands of the dog Malchik, after removal of the cortex of the left cerebral hemisphere. Shaded columns--flow of saliva from the operated side; unshaded columns —flow of saliva from the intact side.

our experiments showed that the secretory function of the gland on the unoperated side is not only not diminished, but is greater than it was before the operation. The general condition of the dogs also gave no evidence of any severe remote sequelae of the operation —the dogs gained weight, and their behavior did not differ from that of normal dogs.

We think that the alterations in the secretory function of the glands can be related to change in their central control following removal of the cortex of one of the cerebral hemispheres. It is noteworthy that unilateral decortication causes the greatest changes in the gland on the operated side, which may be of assistance in locating the cortical areas concerned in the regulation of the secretory function of the salivary glands.

LITERATURE CITED

[1] K. S. Abuladze, Study of the Reflex Activity of the Salivary and Lachrymal Glands,* Moscow, 1953.

[2] V. M. Bekhterev and N. A. Mislavsky, Collected Works of N. A. Mislavsky,* pp. 95-98, Moscow, 1952.

[3] D. A. Biryukov, Byull. Eksptl. Biol. i Med., 5, No. 3, 264-266 (1938).

[4] D. A. Biryukov, Collected Papers on Psychoneurology, dedicated to A. I. Yushchenko,* pp. 123-128 (1928).

[5] O. V. Verneke, Fiziol. Zhur. SSSR, 19, No. 6, 1173-1177 (1935).

[6] I. M. Geiman, Vrach. Gaz., No. 25, p. 608 (1903).

[7] A. V. Gubar and F. A. Oreshuk, Effect of Removal of the Cortex of the Cerebral Hemispheres on the Bile-forming Function of the Liver,* Read at a scientific meeting at the II Moscow State Medical Institute.

[8] I. K. Zhmakin, Effect of Removal of the Cortex of the Cerebral Hemispheres on the Gaseous Metabolism of Dogs,* Thesis, Moscow, 1954.

[9] G. P. Zelenyi, Med. Biol. Zhur. Moscow-Leningrad, No. 1-3, pp. 3-18 (1930).

[10] L. A. Klemenkova and D. S. Fursikov, Transactions of the 3rd All-Union Congress of Physiologists, Biochemists, and Pharmocologists,* p. 65 Leningrad, 1928.

[11] S. L. Levin, Unconditioned Salivary Gland Reflexes in Patients suffering from Brain Tumors and from Traumatic or Vascular Diseases of the Brain,* Thesis, Moscow, 1944.

[12] A. A. Markova, Role of the Cerebral Cortex in Regulation of Gastric Activity,* Read at the Inter-Institue Scientific Therapeutics Congress, Moscow, 1953.

[13] A. P. Savinskaya, Byull. Eksptl. Biol. i Med., 37, No. 2, 7-11 (1954).

[14] Ya. M. Pressman, Sov. Nevropsikhiatriya, Vol. 6 (1941).

[15] Lepine and Bochefontaine, Gaz. Med. de Paris, 1875, N 25.

* In Russian.

EFFECT OF A CIRCUMSCRIBED FOCUS OF INFLAMMATION IN THE BUCCAL CAVITY ON THE "PILOCARPINE" SECRETION OF THE PAROTID GLANDS

A. P. Savinskaya

From the Experimental Pathology Laboratory (Director: Prof. S. I. Lebedinskaya), Department of General Pathology (Director: Academician A. D. Speransky), Institute of Normal and Pathol. Physiology. (Director: V. N. Chernigovsky, Member Acad. Med. Sci. USSR), Acad. Med. Sci. USSR

(Received February 6, 1956. Presented by Academician A. D. Speransky)

Up to the most recent date, textbooks on pharmacology maintain that the secretory effect of pilocarpine is due solely to its action on parasympathetic nerve endings [1, 3, 4, 7].

It was shown, however, by S. I. Gelperin and G. N. Pribytkova [2], and by V. N. Chernigovsky [10] as far back as 1937, that the effect of pilocarpine may vary in the presence of conditioned reflex stimuli, the nature of the alteration in secretion depending on the type of conditioned stimulus applied (negative or positive). O. S. Serebryannikov [9] found that painful stimuli applied during the period of action of pilocarpine may change the nature of the secretion. This finding was confirmed by V. A. Novi [8]. S. L. Levin [6] showed that pilocarpine-induced secretion may undergo alteration during sleep.

We found that when pilocarpine was injected subcutaneously in the mid-cervical line of normal dogs the flow of saliva from the right and the left parotid glands was not uniform.*

We noticed that the uneven secretion of the two parotid glands, in response to pilocarpine injections, was most pronounced in dogs of a "weak" type. The results of a study of pilocarpine-stimulated secretion in normal animals showed that its mechanism followed the same general laws as apply to the secretion of saliva by other unconditioned and conditioned reflex stimuli. The conclusion might hence have been drawn that changes in the functional state of the appropriate nerve centers might be significant factors in determining the nature of the response to introduction of pilocarpine.

In order to test the validity of this conclusion we made a study of the "pilocarpine" secretion of the two parotid glands, in the presence of a circumscribed area of inflammation of the buccal mucosa.

EXPERIMENTAL METHODS

The experiments were performed on two dogs, possessing different types of higher nervous activity, Sedoi being of the weak type, and Ryzhii of the strong.** Medium doses of pilocarpine hydrochloride solution (1.5 ml of 0.1% solution) were injected subcutaneously in the mid-cervical line. Saliva was collected in graduated vessels over the next two hours, the volume being read at 5 minute intervals during this time.

Background secretion was first measured for 9 days, after which we inflicted a burn on the mucous membrane of the gum of the left lower jaw, at the level of the 3rd molar, using a thermocauter with a spherical tip, diameter 0.8 mm.

The inflammatory reaction resulting from the cauterization took a similiar course in both dogs. Immediately after inflicting the burn we saw a damaged area of mucous membrane, with detached shreds of membrane at the margins. During the following 2-4 days a considerable swelling arose around the burnt area, from which the membrane was separating. The mucous membrane around the swelling was of a reddish-purple color. During the following few days a scab formed in the center of the damaged area. The mucosal defect and the swelling gradually diminished, and after the wound had healed (on the 16-21st day) the only remaining effect was that the pigmentation was less then in the surrounding areas.

---

*Uneven secretory response to pilocarpine of the two submaxillary glands was noted by G. N. Kuzmenko [5] in 1940.

**Conditioned reflexes were developed in these animals, and the recognition of the typological peculiarities of their nervous systems was based on prolonged observation of their condition reflex activities, and also on the results of the application of special tests.

EXPERIMENTAL RESULTS

Experiments on the dog Sedoi

Secretion of saliva fell below the normal level on the day the gum was cauterized, from both parotid glands. By the following day secretion had increased considerably on the side of the lesion (left side), but had ceased altogether on the opposite side. On the third day the salivary response to pilocarpine had fallen somewhat on the left side, and had risen on the right. On the 4th day it was totally inhibited on the inflamed side, but again increased with time (on the 5-10th day); see Figure 1.

It thus appears that pilocarpine-stimulated secretion of saliva varied very widely during the first few days following the infliction of the burn. These variations were of a fluctuating nature, and consisted of augmentation or diminution of the flow of saliva, both from the gland on the same side as the inflammatory focus and from that on the opposite side.

Experiments on the dog Ryzhii

On the day the burn was inflicted there was a small rise in the salivary response to pilocarpine, on the same side as the lesion. On the next day secretion on this side had diminished considerably, while that from the opposite gland had fallen to zero. During the succeeding days there was a gradual rise in the response of both glands to pilocarpine injection, to the initial level (Figure 2).

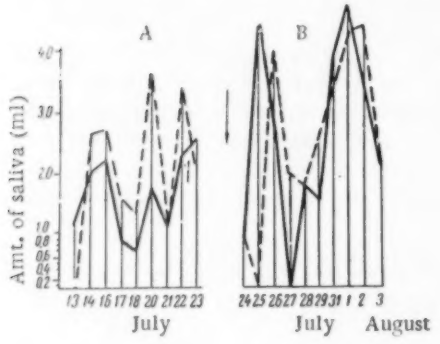


Fig. 1. Flow of saliva, in response to pilocarpine, from the left (continuous line) and right (broken line) parotid glands of the dog Sedoi before (A) and after (B) infliction of a burn (↓) on the gum of the left lower jaw.

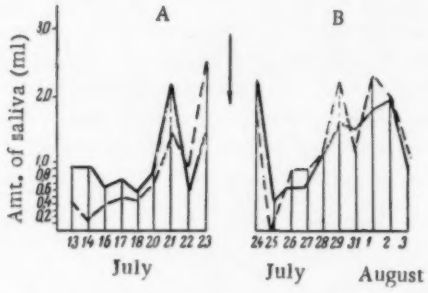


Fig. 2. Flow of saliva, in response to pilocarpine, from the left (continuous line) and right (broken line) parotid glands of the dog Ryzhii before (A) and after (B) infliction of a burn (↓) on the gum of the left lower jaw.

We thus see that the presence of a circumscribed inflammatory focus in the dog Ryzhii caused alteration of the salivary response to pilocarpine of both the parotid glands. These alterations differed, however, from those found for Sedoi, in that they were of short duration, and there was a rapid return to normality.

It is noteworthy that the presence of the inflammatory focus in the buccal muscosa of Ryzhii did not lead to any pronounced asymmetry in the response of the two parotid glands to pilocarpine. The variations in the flow of saliva were in the same direction in the left and the right glands, and any differences observed were of a quantitative nature only. With Sedoi, on the other hand, the secretory action of the two glands was quite unsymmetrical in the presence of the lesion. The maximum rise in secretion by the gland on the same side as the inflammatory focus coincided in time with its total inhibition on the opposite side. Later on, these effects were reversed. Only from the 6th day on did we observe parallel variations in the responses of the two glands, although they still differed considerably in magnitude.

For purposes of comparison, we performed similar experiments on the same dogs, in which the unconditioned reflex stimulus to parotid gland secretion was a meat-biscuit powder meal, instead of pilocarpine.

In these experiments, we waited until the effect on secretion of pilocarpine had ceased (about 2 hours after its injection), and then fed the dogs with a meat-biscuit powder meal (15 g).

The results of these experiments are represented in Figure 3.

The experiments showed that in dogs of a weak type (Sedoi) a circumscribed inflammatory lesion had the same effect on the response to feeding as it did on that to pilocarpine. The changes in secretion in response to a meal were also of a fluctuating nature, and the fluctuations appeared on the same days as did those in response to pilocarpine.

In dogs of a strong type (Ryzhii) the changes in flow of saliva in response to the meat-biscuit powder meal in the presence of a circumscribed inflammatory lesion were restricted to a slight depression of secretion on the opposite side to the lesion.

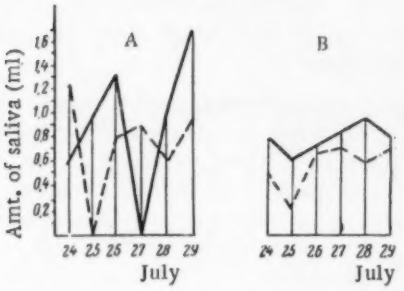


Fig. 3. Flow of saliva, in response to a meat-biscuit powder meal, from the left (continuous line) and right (broken line) parotid glands of the dogs Sedoi (A) and Ryzhii (B), in the presence of an inflammatory lesion of the gum.

It may be concluded that a circumscribed inflammatory lesion of the buccal mucosa affects both reflex secretion of saliva and secretion due to pilocarpine, and the nature of the changes in both types of secretion is in many respects similar. The degree and the nature of these changes are dependent on the typological peculiarities of the nervous system of the subjects.

Our experimental results indicate that the effect produced by pilocarpine is to a considerable extent determined by the functional state of the appropriate nerve centers. This finding merits special attention, inasmuch as up until the present investigations of the action of pilocarpine on secretion have been concentrated chiefly on its effects on the peripheral parts of the nervous system, without taking into account the significance of the initial functional state of the relevant nerve centers.

LITERATURE CITED

[1] N. V. Vershinin, Pharmacology,* 11th Ed., 1953.

[2] S. I. Galperin and G. N. Pribytkova, Research on Neurohumoral Connections,* Compilation edited by K. M. Bykov, No. 3, 1937.

[3] M. I. Gramenitsky, Textbook of Pharmacology,* 3rd Ed., 1941.

[4] N. P. Kravkov, Principles of Pharmacology,* Parts 1-2, 14th Ed., 1933.

[5] G. N. Kuzmenko, Byull. Eksptl. Biol. i Med., 10, No. 6, 457-459 (1940).

[6] S. L. Levin, Byull. Eksptl. Biol. i Med., 36, No. 9, 3 (1953).

[7] M. P. Nikolaev, Textbook of Pharmacology,* 2nd Ed., 1948.

[8] V. A. Novi, Vrachebnoe Delo, 1953, No. 5, 401-404.

[9] O. S. Serebryannikov, Fiziol. Zhur. SSSR, 27, No. 3, 317-321 (1939).

[10] V. N. Chernigovsky, Byull. Eksptl. Biol. i Med., 4, No. 1, 45-46 (1937).

* In Russian.

ROLE OF THE FUNCTIONAL STATE OF THE ALIMENTARY CENTER IN THE MOTOR-EVACUATORY ACTIVITY OF THE STOMACH OF DOGS

I. V. Malkiman and M. A. Vasilevsky

From the Laboratory for the Physiology and Pathology of Digestion (Director: the late I. P. Razenkov, Member Acad. Med. Sci. USSR), Institute of Physiology, Acad. Med. Sci. USSR (Director: Prof. V. N. Chernigovsky, Member Acad. Med. Sci USSR)

(Received November 28, 1955. Presented by V. N. Chernigovsky, Member Acad. Med. Sci. USSR)

In our previous communications we described alterations in the motor-evacuatory activity of the stomach of dogs during the formation and healing of experimental gastric ulcers. The need for the investigation of the mechanisms whereby this function is regulated emerged from a consideration of our findings, since the published literature of this subject is very scanty, and the data are quite contradictory.

It was first shown in I. P. Pavlov's laboratory (Serdyukov) that the acidity of the stomach contents plays a very important part in the mechanism regulating this evacuatory activity. This finding was later confirmed by observations on human subjects, reported by a number of workers [1, 4, 7, and others]. Nevertheless, certain authors [2, 5, 6], especially foreign ones [8], deny the existence of any connection between the acidity of the stomach contents and the speed of their evacuation from the stomach, and consider that the determining factors in the process of evacuation are the mechanical conditions prevailing in the stomach itself; the degree of filling, its peristaltic activity, and the pressure developed in the pyloric antrum.

Very little attention has been paid to the role of the central nervous system in the mechanism of the evacuatory activity of the stomach, and the published data are quite contradictory.

Thus, for example, S. I. Galperin [3] arrives at the conclusion that sham feeding reflexly elicits full or partial closure of the prepyloric sphincter, which would be expected to lead to retardation of evacuation.

On the other hand, Cohnheim and Best [9] found, in experiments on esophagotomized dogs, that sham feeding has the opposite effect, and accelerates evacuation from the stomach of water introduced through a fistula.

In the present research we made a roentgenological study of the role of the functional state of the alimentary center in the mechanism of the evacuatory activity of the stomach.

EXPERIMENTAL METHODS

The experiments were performed on dogs which had been fasted for 18-20 hours. The stomach was washed out before each experiment, until the mucosa gave a neutral reaction. Barium sulfate suspension was introduced, without the knowledge of the dogs, through a gastric fistula, in the same dosage to all the animals (8 g of barium sulfate in 24 ml of water per kg body weight), at the same speed and at constant temperature, applying a small positive pressure. The dog was examined in a horizontal position (lying down), using a tracheoscope, or in a vertical position (standing behind the screen), with the x-rays directed laterally. Care was taken to exclude from the x-ray room any factors which might affect gastric excitability.

X-raying of the stomach was begun while the barium suspension was still being introduced. In order to be able to follow the dynamics of the evacuatory activity of the stomach we took radiograms during the first 3-4 minutes after beginning to introduce the suspension, and at 15 minute, or even shorter, intervals thereafter.

In our experiments we determined: a) the time at which barium began to leave the stomach and enter the duodenum, and b) the way in which evacuation of the stomach contents took place, and the time at which the process was completed.

We performed a series of experiments on each dog, with the object of ascertaining the evacuatory activity of the stomach under ordinary experimental conditions, without the application of outside stimuli (sham feeding, natural conditioned reflex stimuli, etc.).

The experiments were performed on 7 dogs; since the results were the same for all of them, we present those obtained for three of them only, for the purposes of illustration (see Table).

TABLE

Role of the Functional State of the Alimentary Center on the Motor-Evacuatory Activity of the Stomach, in Dogs

Conditions of experiment	Evacuation of stomach contents*										
	Start of passage of Ba into the duodenum (in min)	Amount of barium evacuated during minutes									Time taken for complete emptying of the stomach (in min.)
		5	15	20	30	45	60	82	90	120	180	
Dog Lisa											
Background	0,5	2/3		Traces of Ba left in stomach							25—30
Two minutes of sham feeding	1,5—2		1/2			2/3	4/5		Insignificant residue of Ba in stomach		100—110
Natural conditioned reflex stimulus	1,5—2		2/3		4/5	Traces of Ba left in stomach					48—50
Introduction of histamine	0,5		2/3		4/5		Traces of Ba left in stomach				60—65
Dog Polkan											
Background	0,5	2/3			3/4	Traces of Ba left in stomach					50
Two minutes of sham feeding	1,5—2	1/3			1/2					4/5	Traces of Ba left in stomach	185
Natural conditioned reflex stimulus	1,5—2		1/2		2/3	4/5			Traces of Ba left in stomach			95—100
Introduction of histamine	1,5—2		1/2		2/3	4/5		Traces of Ba left in stomach				90—95

* 8 g of barium sulfate in 24 ml of water were introduced through a gastric fistula, per kg body weight of dog.

TABLE (continued)

Conditions of experiment	Evacuation of stomach contents: Start of passage of Ba into the duodenum (in min.)	Amount of barium evacuated during minutes: 5	15	20	30	45	60	82	90	120	180	Time taken for complete emptying of the stomach (in min.)
Dog Ryzhik												
Background	0,5		3/4		4/5	Insignificant residue of Ba in stomach						55
Two minutes of sham feeding	1,5—2		1/3		1/2		3/4		4/5	Insignificant residue of Ba in stomach		130
Natural conditioned reflex stimulus	1,5—2		1/2		2/3	4/5		Traces of Ba left in stomach				80
Introduction of histamine	1,5—2		2/3		5/6		Insignificant residue of Ba in stomach					65

EXPERIMENTAL RESULTS

Under ordinary conditions, passage of barium suspension into the duodenum begins while it is still being introduced, and the evacuation of the stomach contents proceeds swiftly, especially during the first 15 minutes, during which time over $^2/_3$ of the stomach contents are evacuated. Only insignificant amounts remain in the stomach after 30-50 minutes, and in some animals the stomach has been completely emptied by then.

The following experiments were performed on each of the seven dogs, with the object of elucidating the effect of the functional state of the alimentary center on the motor-evacuatory activity of the stomach. After washing out the stomach, and checking that the mucosa gave a neutral reaction, sham feeding was instituted for 2 minutes, and in most cases radiographic examination of the motor-evacuatory activity of the stomach was commenced as soon as an acid reaction of the gastric mucosa was perceived.

The results (see Table) showed that under these conditions evacuation of the stomach contents is considerably retarded, lasting for 120-180 minutes (i.e., 2-3 times slower than usual). The nature of the process also changes: it proceeds more uniformly, not being faster to begin with. Passage of barium into the duodenum does not take place immediately on its introduction, but only $1^1/_2$ - 2 minutes later.

In the next series of experiments we attempted to ascertain to what extent this alteration in the motor-evacuatory activity of the stomach due to sham feeding was dependent on stimulation of the cortical alimentary center.

With this object, we stimulated the alimentary center of all seven dogs, applying a natural conditioned stimulus.

After washing out the stomach to a neutral reaction of its mucosa, we cut a lump of meat up into pieces for 4-5 minutes, in sight of the dog, as if in preparation for feeding it. The reaction of the gastric mucosa usually became acid after 5-7 minutes, and secretion of gastric juice began; at this moment we proceeded to the radiographic observation of the motor-evacuatory activity of the stomach.

We found from these experiments that the evacuation of the stomach contents was considerably retarded, and proceeded more uniformly than when the barium suspension was introduced through the fistula without preliminary stimulation of the alimentary center.

Passage of barium from the stomach into the duodenum does not begin at once, but $1\frac{1}{2}$-2 minutes after introduction of the suspension into the stomach was commenced. The alterations in the motor-evacuatory activity of the stomach were basically the same as after sham feeding, although they were less pronounced (see Table).

The differences in evacuation times after sham feeding and after stimulation with a natural conditioned stimulus are ascribable to the summation of exteroceptive impulses and impulses originating in the buccal cavity in the former case, leading to a more intense stimulation of the alimentary center.

It should also be remembered that the secretory responses of the gastric glands to natural conditioned stimuli is much weaker than that due to sham feeding. It lasts for only 45-50 minutes, the amount of gastric juice secreted varies from 5-17 ml (whereas with sham feeding a mean secretion of 45-50 ml is found during the first hour alone, and secretion persists for 2-3 hours), and the acidity is very low. Free acid is either absent, or amounts to no more than 0.12, as compared with 0.4-0.5 with sham feeding.

The differences between the evacuation times for sham feeding and natural conditioned stimulation may hence also be ascribed, to a certain extent, to the differences in the amount of secretion in the former case, and in its acidity in the latter.

We have referred above to the work of Serdyukov, of I. P. Pavlov's laboratory, who first showed that hydrochloric acid plays an important part in the motor-evacuatory activity of the stomach of dogs. We therefore proceeded to investigate the extent of the effect of gastric secretion in sham feeding and in response to a natural conditioned stimulus, on the motor-evacuatory acitivy of the stomach.

With this object, we performed a series of experiments in which subcutaneous histamine injections were given to the dogs.

We first determined the amount and the acidity of gastric secretion produced by the dogs in response to sham feeding for 2 minutes. We then injected a dose of histamine, such that it caused about the same secretory effect in a given dog as did the sham feeding.

After washing out the stomach until the mucosa gave a neutral reaction, we injected the appropriate dose of histamine subcutaneously, and 3-5 minutes after gastric secretion had begun we filled the stomach with barium suspension, and proceeded immediately to the radiographic recording of gastric motor-evacuatory activity.

An examination of the results shows that passage of stomach contents into the duodenum commenced during the first 1-$1\frac{1}{2}$ minutes (rarely towards the end of the second minute) after beginning to introduce barium suspension. Evacuation of stomach contents was retarded, although to a much smaller extent than with sham feeding, although the amount of gastric juice secreted was about the same as in sham feeding, and in some cases even larger.

Our findings permit of the following conclusions. The motor-evacuatory activity of the stomach is a complex and coordinated process, in the regulation of which a quite important part is played by the functional state of the alimentary center, reflex and conditioned reflex stimulation of which causes considerable retardation of evacuation.

LITERATURE CITED

[1] Yu. P. Arkussky, Vestnik Rentgenol. i Radiol., 7, No. 2, 111-117 (1929).

[2] M. E. Vasilevsky, Med. Zhur. BSSR, 1941, No. 13, 19-27.

[3] S. I. Galperin , Arkhiv Med. Nauk, 2, No. 2-3, 229-243 (1929).

[4] L. M. Mailyan, Azerb. Med. Zhur., 1938, No. 2, 115-120.

[5] I. A. Oksenov, Vestnik Rentgenol. i Radiol., 3, No. 1, 61-63 (1924).

[6] I. A. Oksenov, Vestnik Rentgenol. i Radiol., 3, No. 1, 64-66 (1924).

[7] U. A. Edelman, Gastric Movements and Passage of Stomach Contents into the Intestines,* 1906.

[8] C. W. McClure, L. Reynolds and C. O. Swartz, Arch. Int. Med. Chicago, 1920, Vol. 26, pp. 410-423.

[9] Conheim and Best, Munch. med. Wschr., 1910, N. 35, S. 1858.

EFFECT OF STIMULATION OF THE HYPOTHALAMUS ON THE MOTOR-SECRETORY ACTIVITY OF THE EMPTY STOMACH, IN EXTENSIVE EXPERIMENTS ON DOGS

A. F. Kosenko

From the Chair of Human and Animal Physiology (Director: Prof. A. I. Emchenko), Kiev State University

(Received January 2, 1956. Presented by N. S. Kupalov, Member Acad. Med. Sci. USSR)

The effect of stimulation of the hypothalamus on the periodic activity of the fasting stomach has been relatively little studied. Most of the work done was in conditions of short experiments, and the results obtained were contradictory. We have been unable to find any references in the literature to the effect of hypothalamic stimulation on gastric secretion in extensive experiments.

Some of the published work has been on animals in which the hypothalamus had been destroyed. N. N. Burdenko and B. N. Mogilnitzki [7] found that destruction of the hypothalamus in dogs was followed by an increase in the amount and the acidity of the gastric juice. Beattie [8] found that stimulation of the lateral wall of the hypophyseal infundibulum of cats under light barbiturate narcosis was followed by increased peristalsis and gastric secretion.

I. I. Burachevsky [1] studied the periodic activity of the empty stomach after damaging the tuber cinereum. He found no significant difference following this operation, as compared with normal animals.

G. M. Davydov [2] found that lesions of the region of the tuber cinereum caused increased gastric secretion in dogs.

G. Bodechtel and O. Kaufmann [9] observed increased peristalsis after stimulating the anterior part of the hypothalamus, whereas E. Gelgorn [3] found the opposite effect.

Rusishvili (1949) found that lesions of the hypothalamic region of dogs caused disturbances in the periodic activity of the stomach and in the spontaneous secretion of gastric juice. I. A. Chereshnev [5] showed that stimulation of the hypothalamus of dogs delayed evacuation of gastric contents into the duodenum.

G. and B. Uvnas [10] found that stimulation of the frontal lobes, basal nuclei, and hypothalamus of cats (acute experiments) was followed after 30-40 seconds by contraction of the stomach.

From all these data it might be concluded that stimulation of the anterior parts of the hypothalamus causes motor and secretory activity of the stomach, which are inhibited by stimulation of the posterior parts. We have attempted, using improved methods and in extensive experiments, to investigate the effect of hypothalamic stimulation in dogs on the motor-secretory activity of the empty stomach.

* In Russian.

EXPERIMENTAL METHODS

The experiments were performed on fasting dogs with a permanent Basov gastric fistula. We examined the motor and secretory activities of the dogs 6-8 days after the operation. After recording the background activities over a period of 30 days we inserted an indwelling electrode, for the purpose of stimulating the hypothalamus in extensive experiments. The electrodes, mounted on an arcuate piece of Plexiglass, were placed around the infundibulum, so that one pair was located at the anterior, and the other at the posterior, part of the hypothalamus. The technique of implanting the electrodes was worked out by us together with P. G. Bogach [6]. Stimulation of the hypothalamus was achieved by means of a sonic generator, Type GZ-1, with a frequency of 20 to 200cps, and an amplitude of 15-20 mv.

Stomach movements were recorded on a kymograph, using air-water transmission from a rubber bag, and a Marey capsule. Gastric juice was collected in a graduated cylinder, and its free and combined acid content and digestive power (according to Metta) are determined.

During 1-2 hours before each experiment we recorded the periodic activities of the stomach without hypothalamic stimulation, and collected gastric juice. After determining the background conditions we proceeded to stimulate the hypothalamus.

EXPERIMENTAL RESULTS

The experiments were performed on two dogs.

Bobik, a male farmyard dog weighing 12 kg, was operated for a Basov fistula on May 30, 1955. Observations were begun 13 days after the operation and lasted for 30 days.

Four polar electrodes were placed on the hypothalamus on July 1, 1955. The experiments began again on the 6th day after the operation, and lasted until October 18, 1955.

During the first two experiments we recorded stomach contractions in the absence of hypothalamic stimulation. We found that "acid" movements took place continuously during the whole experiment. Later on, the periodic activity of the stomach became of the normal type, not differing from that observed before the operation. We then proceeded with the experiments involving stimulation of the hypothalamus.

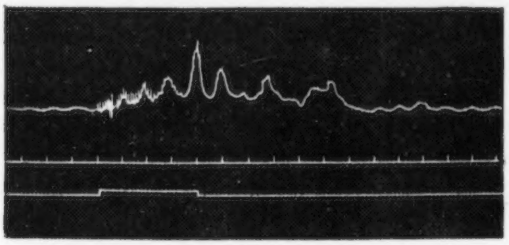


Fig. 1. Heightening of tonus, and appearance of gastric contractions due to stimulation of the hypothalamus (anterior parts) during a phase of gastric inactivity, for the dog Sultan. Explanation of tracings (from above down): recording of stomach movements, time signals (15 sec.), and stimulation signal (15 cps, 15 A).

In all cases, stimulation of the posterior hypothalamus of Bobik against a background of gastric activity caused lowering of tonus and inhibition of periodic contractions, both in the pyloric part and in the fundus of the stomach; in addition, we observed widening of the palpebral fissures, contractions of the pupils, and panting. The dog raised his head and sniffed the air.

During a quiescent phase of gastric activity, stimulation by the posterior electrodes did not cause any motor effects. Stimulation by the anterior electrodes during a period of inactivity raised the tonus of the stomach, and

was followed, after a latent period of 10-15 seconds, by gastric contractions. At the same time we observed narrowing of the palpebral fissures and the pupils and acceleration of the rate of breathing. The dog started to lick himself. Stimulation by the anterior electrodes while the stomach was active caused slight raising of tonus, with increased amplitude of the individual contractions.

Before the electrodes were implanted, the gastric juice collected over a 4-5 hour period amounted to about 8 ml, with a free acid content of 0-10, a total acidity of 2-20, and a digestive power of 0 to 1 mm.

In the first two experiments performed after implanting the electrodes we collected up to 70 ml of gastric juice over the same time, with a free acid content of 50 to 120, a total acidity of 100 to 140, and a digestive power of 2 to 6 mm. On the third day after the operation gastric secretion had returned to preoperational levels.

With hypothalamic stimulation we collected, over 4-5 hours time, 3 to 20 ml of gastric juice, with a free hydrochloric acid content of 20 to 50, total acidity of 30 to 105, and a digestive power of 1 to 3 mm of protein.

The stomach contractions and secretion observed in experiments in which we did not stimulate the hypothalamus were of the same order as before implantation of the electrodes.

Our second experimental dog, Sultan, (a watchdog, weight 23 kg, male) was given a gastric fistula on June 15, 1955. After 7 days we began to take recordings, which lasted for a month, to July 16, when 4 polar electrodes were implanted in the hypothalamic region. After 14 days we renewed our experiments, which lasted until October 24, 1955.

No perceptible effect resulted from stimulation from the anterior and posterior electrodes while the stomach was working. Stimulation during a quiescent period caused contractions, with a latent period of 15-35 seconds (duration of stimulation 1 minute; see Figure 1).

In all, we performed 300 such stimulations during our experiments. We always found copious secretion of gastric juice, with heightened free and total acidity, and raised digestive power. These effects followed both single stimulations and stimulations repeated at 30 minute intervals during the whole experiment. The results of two experiments are represented in Figures 2 and 3.

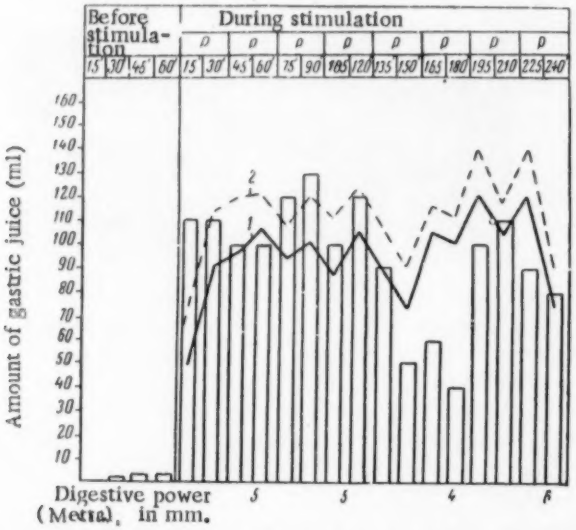


Fig. 2. Alterations in gastric secretion due to stimulation of the hypothalamus for 1 minute with a current frequency 30 cps, amplitude 15 mv, at 30 minute intervals. 1) Free hydrochloric acid, in Ewald units; 2) total acidity in Ewald units; average digestive power of a 1 hour portion of gastric juice (Metta), in mm of protein digested in 24 hours (figures below). Experiment on Sultan, July 30, 1955.

Other effects noticed during stimulation from the anterior electrodes, in the dog Sultan, were defecation, urination, and narrowing of the pupils, and the dog licked himself. Stimulation from the anterior electrodes caused dilatation of the pupils, with convergent squint, and the dog sniffed the air.

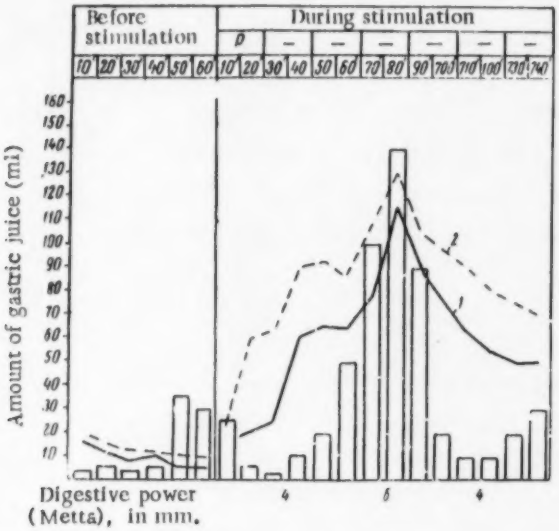


Fig. 3. Alterations in gastric secretion with a single stimulation of the hypothalamus (current frequency 50 cps, amplitude 15 mv, duration 1 minute). 1) Free hydrochloric acid, in Ewald units; 2) total acidity in Ewald units; columns: ml of gastric juice per 10 minutes; average digestive power of a 40 minute portion of gastric juice, in mm of a protein column digested in 24 hours (Metta test; figures below). Experiment on Sultan, September 2, 1955.

Before implantation of the electrodes secretion of gastric juice over 4-5 hours amounted to 2 to 10 ml, of a free acid content of 0 to 15, total acidity 2 to 20, and digestive power of 0 to 1 mm.

During stimulation of either the anterior or the posterior hypothalamus the amount of juice collected over the same time was 40 to 150 ml, with a free hydrochloric acid content of 30 to 144, a total acidity of 40 to 160, and a digestive power of 2 to 7 mm.

We found that the maximum effect was achieved when the hypothalamus was stimulated with a current of frequency 30 to 80 cps, and amplitude 15-20 mv.

Thus we see that stimulation of the hypothalamic region, in extensive experiments on dogs, causes alterations in the motor activity of the fasting stomach; appearance of untimely contractions of the stomach during a quiescent period, when the anterior part of the hypothalamus is stimulated, and inhibition of rhythmic contractions during a phase of gastric activity when the posterior part is stimulated. Stimulation from both the anteriorly and the posteriorly placed electrodes raises the secretion of gastric juice, of high acidity and considerable digestive power, and also causes defecation, urination, panting, and dilatation of the pupils.

LITERATURE CITED

[1] I. I. Burachevsky, Problemy Endokrinol., 1936, No. 1, 32-39.

[2] G. M. Davydov, Researches on the Physiology and Pathophysiology of the Human Digestive Apparatus,* Vol. 4, pp. 243-254, Moscow, 1938.

[3] E. Gelgorn, Regulatory Functions of the Autonomic Nervous System,* p. 206 (1940).

* In Russian.

[4] M. B. Tetyaeva, Jubilee Scientific Meeting in Honor of the Centenary of the Birth of I. P. Pavlov,* Papers presented, pp. 135-137, Moscow-Leningrad, 1949.

[5] I. A. Chereshnev, Byull. Eksptl. Biol. i Med., 29, No. 6, 429-433 (1950).

[6] P. G. Bogach and A. F. Kosenko, XII Scientific Congress of the T. G. Shevchenko Kiev State University,* pp. 134-136, Kiev, 1955.

[7] N. Burdenko and B. Mogilnitzki, Z. ges. Neurol. Psychiat., 1926, Bd. 103, H. 1-2, S. 42-62.

[8] J. Beattie, Can. Med. Assoc. J., 1932, Vol. 26, pp. 400-405.

[9] G. Bodechtel and O. Kaufmann, Fortschr. Neurol. Psychiat. u Grenzgebiete, 1938, H. 2, S. 52-73.

[10] G. Strom and B. Uvnas, Acta physiol. Scandinav, 1950, Vol. 21, pp. 90-104.

EFFECT OF NATURAL AND DRUG-INDUCED SLEEP ON GASTRIC SECRETION IN DOGS

N. Sh. Amirov

From the Laboratory for the Physiology and Pathology of Digestion (Director: Dr. Med. Sci. S. I. Filippovich), Institute of Normal Pathol. Physiology (Director: Prof. V. N. Chernigovsky, Member Acad. Med. Sci. USSR), Acad Med Sci. USSR

(Received April 1, 1956. Presented by V. N. Chernigovsky, Member Acad. Med. Sci. USSR)

Drug-induced sleep has been widely applied in therapeutics over the past few years, in particular in the treatment of gastric ulcer. It is known that the disturbances in gastric secretion encountered in ulcerative disease play a certain part in its pathogenesis and in its recurrence. Clinical data on the effect of drug-induced sleep on gastric secretion in ulcer patients are very contradictory, owing to the differences between the test methods used, and because of the use of diverse hypnotics, with different modes of action.

Not enough work has been done on the theoretical basis of the indications or counter-indications for the application of drug-induced sleep to the treatment of functional disturbances of the gastrointestinal tract.

The object of our research was to investigate the effects of natural and drug-induced sleep on the secretory activities of the gastric glands.

EXPERIMENTAL METHODS

The hypnotics used were Barbamyl and chloral hydrate. The research was conducted under the conditions of extensive experiments. The experiments were performed on 10 dogs with Pavlov and Klemensievich-Heidenhain gastric pouches, and with gastroesophagotomies. Barbamyl was introduced rectally at a dosage of 0.04 g per kg body weight, and chloral hydrate at a dosage level of 0.3-0.4 g per kg body weight. In view of the intense local irritation caused by chloral hydrate solution we dissolved it in starch solution. At the given dosage levels, Barbamyl induced sleep lasted for 7-8 hours, and chloral hydrate sleep for 3-4 hours. Immediately after administration of the hypnotic, and before it had had time to act, the dogs were given a carefully measured amount of gastric stimulant (hematogen, raw meat, rye bread).

The experiments involving the use of hypnotics were preceded by experiments designed to determine the normal secretory response of the gastric glands to the stimulants, in wakefulness and in natural sleep. The latter experiments were performed at night, after gastric stimulation in a darkened room, as near as possible soundproof.

* In Russian.

We recorded changes in the amount of secretion, and in its free HCl content and total acidity, as well as in its digestive power (according to Metta). We investigated the effects of both single and multiple doses of hypnotic (intermittent sleep over 5 days), in order to elucidate the action and the after-effects of prolonged drug-induced sleep. During the experiments the dogs were accommodated in specially constructed cradles.

EXPERIMENTAL RESULTS

A considerable diminution in the flow of gastric juice was observed during the first few hours of natural sleep (Figure 1) in dogs provided with a Pavlov gastric pouch, and also with sham feeding. Natural sleep caused no perceptible alterations in the flow of gastric juice in dogs with a Heidenhain pouch. It may be concluded that natural sleep depresses gastric secretion in its complex reflex phase, but has no perceptible effect in its neurochemical phase.

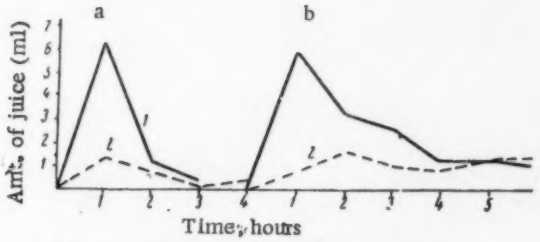


Fig. 1. Gastric secretion of dogs with a Pavlov pouch during wakefulness and during natural sleep. a) Stimulus: 100 g of rye bread; b) stimulus: 200 g of raw meat; 1) when awake; 2) during sleep.

Administration of Barbamyl caused a considerable reduction in gastric secretion during the first hours of sleep, both in dogs with a Pavlov pouch, and after sham feeding of gastroesophagotomized dogs (Figure 2).

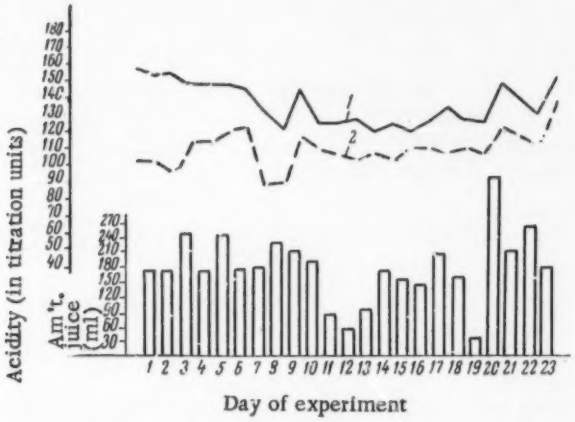


Fig. 2. Gastric secretion in a gastroesophagotomized dog after sham feeding during natural and drug-induced sleep. 1) 10th, 13-18th, and 20th-23rd day: secretion after sham feeding during wakefulness; 11th day: the same during natural sleep; 12th and 19th day: during drug-induced sleep; 1) total acidity; 2) free HCl.

We found certain after-effects of intermittent sleep lasting for 5 days, caused by administration of single daily doses of Barbamyl to dogs with a Pavlov gastric pouch; the amount of gastric juice collected fell during 20-25 days, in the first phase of secretion. In certain cases we found that Barbamyl induced sleep was associated with a fall in the acidity and digestive power of the gastric juice; these variations appear to be connected with differences in the depth of drug-induced sleep in different experiments. No particular changes were seen in the amount of juice secreted by dogs with a Heidenhain pouch, but a prolongation of secretion time was found in all cases. The time taken to secrete a given amount of gastric juice in response to a measured dose of hematogen rose from the normal value of 2 hours to 3 hours during sleep.

Our experiments showed a considerable fall in secretion extending over the entire duration of sleep induced by chloral hydrate in Pavlov pouch dogs, i. e., there is a depression of both the complex reflex and the neurochemical phases.

Administration of chloral hydrate was followed by diminution of secretion of gastric juice in both Pavlov and Heidenhain pouch dogs, as well as in response to sham feeding of gastroesophagotomized dogs.

In general, the higher the dose of chloral hydrate given, the greater was the subsequent fall in gastric secretion; this fall was associated with a steep fall in the acidity of the juice (Figure 3).

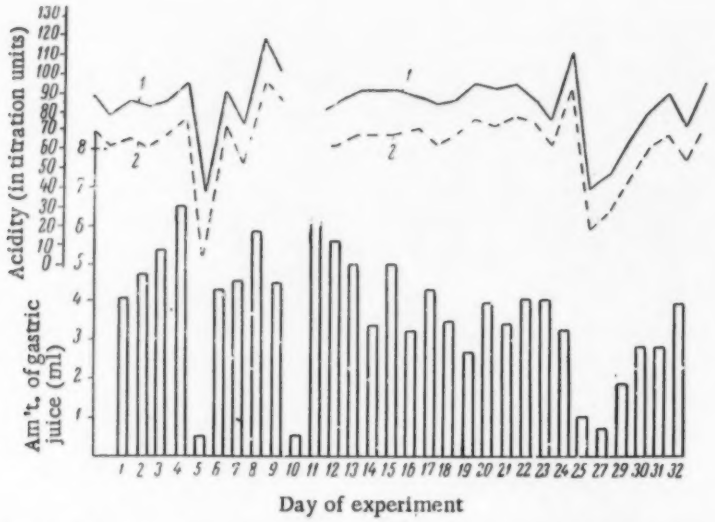


Fig. 3. Gastric secretion during drug-induced sleep, in dogs with a Heidenhain pouch. 1st-4th, 6-9th, 11-24th, and 30th to 32nd days: secretion during wakefulness; 5th, 10th, and 25-29th days: secretion during drug-induced sleep. 1) total acidity, 2) free HCl.

Diminished flow of gastric juice persisted for 20-25th days after 5 days of intermittent sleep induced in Heidenhain pouch dogs by single daily doses of chloral hydrate (Figure 3).

We may thus conclude that the alterations in gastric secretion under the influence of chloral hydrate are the result of a complex combination of its effect on the central nervous system and on the peripheral neuroglandular apparatus of the stomach.

Our results indicate that the amount of gastric juice secreted in the complex reflex phase falls during both natural sleep and during sleep induced by Barbamyl or chloral hydrate. Each of these hypnotics, however, exerts its own specific effect: Barbamyl causes a certain prolongation of secretion time; chloral hydrate causes a considerable fall in the amount secreted by a Heidenhain pouch dog. Barbamyl exerts an after-efect—a diminution in the amount of gastric juice produced in the complex reflex phase of secretion; chloral hydrate shows this effect in Heidenhain pouch dogs.

The results presented above may be of significance in establishing indications or counter-indications for applying drug-induced sleep therapy, in particular with reference to a hyposecretory state of the stomach.

INHIBITORY ACTION OF RIBONUCLEASE ON SECRETION OF HYDROCHLORIC ACID BY SURVIVING FROG'S GASTRIC MUCOSA

Ya. A. Epshtein

From the Chair of Biochemistry, Abu ali ibn Sina Stalinabad Medical Institute

(Received June 27, 1956. Presented by V. A. Engelhardt, Member Acad. Med. Sci. USSR)

The role of nucleic acids, and in particular of ribonucleic acid (RNA), in cell metabolism has been elucidated by the work of M. Brachet [1], Casperson [17, 18], B. Kedrovsky [6, 8], and others. M. Guberniev, I. Kovyrev, and L. Ilina [4, 5] have shown the connection between RNA and the processes of synthesis of digestive enzymes. The important part played by RNA in the functioning of the glands of the alimentary tract has been established by Casperson [17, 18], M. Deken-Grenson [19], and others. The discovery by us of the inhibitory effect of basic dyes on hydrochloric acid secretion [12] led us to the conclusion that, since these dyes combine with nucleic acids, their effect is due to direct blocking of the cyclophorase system, the basic component of which is RNA. D. Yakhina [14] has shown that the RNA activity of the gastric mucosa rises considerably during heightened secretion of hydrochloric acid. Thus the participation of RNA has been demonstrated not only in the synthesis of digestive enzymes, but also in the process of secretion of hydrochloric acid. The active participation of RNA in the secretion of amylase by the pancreas, although not in the process of synthesis of this enzyme, has been shown by L. Hokin [20-22]. A number of recently published papers have shown that protein synthesis and RNA activity do not always vary parallel [19, 23].

Thus, there is every reason to believe that RNA takes part in the accumulation of high-energy phosphate bonds [2, 3, 27], and thus provides for the normal vital activities of cells.

As far back as 1951 we performed experiments on the action of ribonuclease on secreting surviving gastric mucosa of frogs. These experiments were based on Brachet's finding that this specific enzyme is capable of penetration into fixed tissue sections, so serving for the histochemical identification of RNA in tissues. At that time we had no reason to believe that ribonuclease, which has a molecular weight of the order of 12,000-15,000, is able to penetrate into living tissues, although it had a pronounced inhibitory effect on secretion of HCl by surviving gastric mucosa.

It has been shown in the recent papers of J. Brachet [15, 16] and L. Ledoux [24, 25] that ribonuclease is able to penetrate freely into living tissues. This has led us to publish our findings on the inhibitory action of ribonuclease on secretion of HCl by surviving gastric mucosa of frogs.

The opinion has been expressed by some authors working on ribionuclease that it may exert a proteolytic a action, owing to its admixture with proteases. This possibility is eliminated if the ribonuclease is prepared according to M. McDonald [26]. We cite below the findings of Yakhina on the action of salivary ribonuclease on secreting surviving frog's gastric mucosa, which exclude the possibility of any such side action of the enzymatic preparation, since it is known that proteolytic enzymes are absent from the saliva. Thermal treatment of saliva has been shown by G. I. Roskin [11] to assure the action of ribonuclease on histological tissue sections, invariably, and beyond any doubt. Ledoux [24, 25] has demonstrated that direct introduction of some proteolytic enzymes into living cells has no effect on cell division.

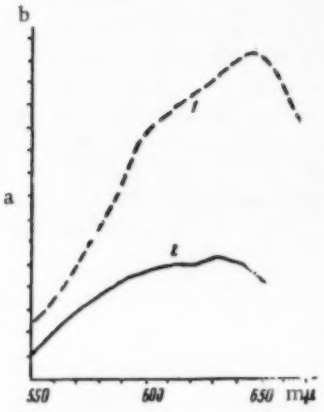
EXPERIMENTAL METHODS

Surviving frog's gastric mucosa was prepared and used for experiment in the way described in a previous paper [12]. To the nutrient medium we added active crystalline ribonuclease, provided by M. V. Pavlova, or else human saliva (1 ml) which had been heated at 70° for 30 minutes; 25 ml of nutrient medium, prepared according to Yakhina, was taken. In order to exclude any possible effects due to ammonium salts used to precipitate ribonuclease, we first removed ammonium ions by passing the enzyme solution through a phenosulfonic cationite in the hydrogen phase.

The amount of hydrochloric acid produced by the surviving tissue during 4 hours was determined by conductometric titration [12]. In some of the experiments we added 1 ml of 0.05% toluidine blue to the medium, in order to check the vitality of the cells, and in others we stained pieces of tissue with the same solution, after completion of the experiment. Living tissues are able to decolorize toluidine blue, changing it into the leuco-form. Apart from this, RNA is able to shift the absorption maximum of toluidine blue, from 630 mμ in its absence to 650 mμ in its presence (see Figure). As a result of this shift, the dye changes color from blue to green. This bathochromic effect is not given by RNA after depolymerization with ribonuclease.

EXPERIMENTAL RESULTS

Our experiments showed that surviving mucosa becomes decolorized if the access to air is restricted (under a cover slip), and that it acquires a violet coloration as soon as the cover slip is lifted. Tissue treated with cyanides or with alcohol immediately acquires a permanent blue coloration. Microscopic examination showed that the toluidine blue stained parts had a greenish tinge in places, whereas after the action of ribonuclease the color changed to blue, approaching a violet tinge.



Absorption spectrum of toluidine blue in the presence of ribonucleic acid (1), and in its absence (2).

The results of experiments on the secretion of hydrochloric acid by frog's surviving gastric mucosa are presented in the Table.

As appears from the Table, ribonuclease powerfully, and in some cases totally, inhibits secretion of HCl by surviving gastric mucosa. This effect can only be explained if we assume that ribonuclease penetrates into the cells, and blocks the biochemical processes responsible for the normal secretion of HCl. Thus, our supposition that RNA plays an essential part in the process of secretion or formation of HCl in the gastric mucosa [13, 14] is confirmed by the direct action of active ribonuclease on RNA.

Enhanced secretion of HCl by surviving gastric mucosa, caused by addition of histamine, is inhibited by addition of ribonuclease to the nutrient medium. This effect of ribonuclease on the activity of surviving gastric mucosa confirms our previously expressed assumptions.

Amount of HCl, in μM per sq. cm of Surviving Gastric Mucosa Produced in 4 Hours in an Aerated Nutrient Solution, in the Absence and the Presence of Active Ribonuclease

Control HCl, μM/sq. cm	Crystalline ribonuclease + + HCl, μM/sq. cm	Saliva* + HCl, μM/sq. cm
Mean of 23 experiments 4.47 ± 0.30	Mean of 7 experiments 2.4 ± 0.40	In 5 experiments 0.00 In one experiment 0.47 In one experiment 1.57 In one experiment 2.97 In one experiment 3.14

* The data of this column are taken from Yakhina.

LITERATURE CITED

[1] J. Brachet, Uspekhi Sovrem. Biol., 29, 140-144 (1950).

[2] S. E. Bresler and E. I. Nidzyan, Doklady Akad. Nauk SSSR, 75, 79-82 (1950).

[3] S. E. Bresler and Kh. M. Rybina, Biokhimiya, 20, 740-748 (1955).

[4] M. A. Guberniev and I. G. Kovyrev, Problems of Medical Chemistry,* Vol. 1, No. 1-2, pp. 363-368 (1949).

[5] M. A. Guberniev and L. I. Ilina , Doklady Akad. Nauk SSSR, 71, 351-353 (1950).

[6] B. V. Kedrovsky, Biol. Zhur., 6, No. 5-6, 1137-1198 (1937).

[7] B. V. Kedrovsky, Uspekhi Sovrem. Biol., 15, 295 (1942).

[8] B. V. Kedrovsky, Uspekhi Sovrem. Biol., 31, 38 (1951).

[9] M. V. Pavlova, Doklady Akad. Nauk SSSR, 92, 641-643 (1953).

[10] G. V. Titova and V. S. Shapot, Biokhimiya, 20, No. 4, 485-489 (1955).

[11] G. I. Roskin, Microscopy Techniques,* Moscow, 1951.

[12] Ya. A. Epshtein and G. V. Mukha, Biokhimiya, 17, No. 4, 392-402 (1952).

[13] Ya. A. Epshtein and L. A. Aleksandrova, Biokhimiya, 18, 701-705 (1953).

[14] D. N. Yakhina, Biokhimiya, 21, No. 3, 425-433 (1956).

[15] J. Brachet, Nature, 1954, Vol. 174, pp. 876-877.

[16] J. Brachet, Nature, 1955, Vol. 175, pp. 851-853.

[17] T. Casperson, Naturwissenchaften, 1941, H. 3, S. 32-43.

[18] T. Casperson, Landstrom-Hyden, and L. Aquilonius, Chromosoma, 1941, Vol. 2, pp. 111-131.

[19] M. De Deken-Grenson, Bioch. Bioph. Acta, 1953, Vol. 12, pp. 560-569.

[20] L. E. Hokin, Bioch. Bioph. Acta, 1952, Vol. 8, p. 225.

[21] M. P. Hokin, J. Biol. Chem., 1953, Vol. 203, pp. 967-977.

[22] Ibid, Bioch. Bioph. Acta, 1954, Vol. 13, pp. 401-412.

[23] R. Jeener, Bioch. Bioph. Acta, 1950, Vol. 8, pp. 270-282.

[24] L. Ledoux, Nature, 1955, Vol. 175, pp. 258-259.

[25] L. Ledoux, C. J. LeClerc and F. Vandenhaeghe, Nature, 1954, Vol. 174, pp. 793-794.

[26] M. R. McDonald, J. Gen. Physiol., 1948, Vol. 32, pp. 39-42.

[27] S. Spiegelmann and M. D. Kamen, Science, 1946, Vol. 104, pp. 581-584.

EFFECT OF INTRAVENOUS INJECTION OF HETEROLOGOUS SERUM ON GASTRIC SECRETION

N. E. Dukler

From the Chair of Biochemistry (Director: Prof. N. P. Pyatnitsky , Kuban Medical Institute

(Received April 21, 1956. Presented by V. N. Chernigovsky, Member Acad. Med. Sci. USSR)

It has been shown by I. P. Razenkov [5] and his co-workers that proteins and other nitrogenous substances are secreted with the gastric juice.

* In Russian.

Similar findings were reported by V. M. Rubel et al. [6], following transfusions into patients of whole blood, serum, or colloidal extenders. The amount of gastric juice rose after transfusions, while its chloride and pepsin contents fell. In most cases the total nitrogen content of the gastric juice rose after transfusions. This increase was due not only to increase in protein and polypeptide nitrogen, but also in nonprotein nitrogen.

It was, in this connection, of interest to ascertain the effect of intravenous injection of heterologous protein on the secretory function of the gastric glands, and to see whether injection of heterologous serum augmented secretion of nitrogenous substances in the gastric juice.

The effect on gastric secretion of injection of heterologous proteins has become of particular interest, in view of the use as blood extenders and parenterally administered nutrients of a number of preparations containing denatured and hydrolyzed heterologous proteins (N. G. Belenky [1], N. A. Fedorov, G. A. Tsinman). Apart from this, the prophylaxis and treatment of a number of infectious diseases are associated with introduction of heterologous proteins into the organism.

EXPERIMENTAL METHODS

Our experiments were performed on 5 dogs, with gastric fistulae: Laska, weight 14.5 kg, a lively dog, eats greedily, rather nervous; Druzhok, 15.5 kg, a quiet dog, does not eat greedily; Tobik, 16.8 kg, lively, eats greedily; Palma, 14.3 kg, active, eats greedily; Bobik, 15 kg, moderately active, eats greedily.

We used sterile bull's serum as the heterologous protein.

Gastric secretion was stimulated by subcutaneous injection of morphine into dogs which had been fasted for 20 hours. The stomach was washed out with warm water before injection of morphine.

It has been shown by A. I. Smirnov and V. F. Shirokov [8] that subcutaneous injection of 0.005-0.01 g of morphine into fasting dogs causes a copious secretion of gastric juice. According to E. G. Lipskaya [3] the chemical composition and pepsin content of this juice is practically identical with that secreted in response to histamine.

The technique adopted in our experiments provided us with considerable amounts of gastric juice, free of food residues. The dosages used were: Laska, Tobik, and Bobik 0.01 g, Druzhok and Palma 0.005 g of morphine hydrochloride.

The gastric juice secreted after injection of morphine was collected at the end of each hour, and was subjected to the following analyses: acidity according to Michaelis, enzymatic activity, from its milk-curdling power, in Pyatnitsky units [4], and nitrogen, by the Kjeldahl method.

After having determined the properties of the gastric juice secreted in response to morphine, we proceeded to inject bull's serum into dogs during the phase of gastric secretion induced by morphine. Two injections were given to each dog; the first of 20 ml, and the second, four days later, of 30 ml.

EXPERIMENTAL RESULTS

The dogs were unaffected by the first injection. After the second injection Laska showed obvious signs of nasal congestion.

The amount of gastric juice secreted (mean hourly figure) by four dogs in response to morphine fell after injection of bull's serum (Figure 1). The total hydrochloric acid content fell for the first four dogs (Figure 2).

The total nitrogen content (Figure 3) of the gastric juice was, for Laska, 14.3 mg per 1 hour portion after morphine alone, 22.2 mg after the first injection of serum, and 4.4 mg after the second. The corresponding values for the other 4 dogs were:

Palma: 12.5, 16.5, and 17.6 mg,
Bobik: 4.4, 11.8, and 17.3 mg,
Tobik: 9.8, 12.8, and 11.5 mg,
Druzhok: 13.7, 10.7, and 11.6 mg, respectively.

The pepsin content of the gastric juice fell, for two dogs, after the first and second injections of bull's serum. The mean pepsin content of a 1 hour portion of gastric juice from Laska was: morphine alone, 52.6,

first serum injection 52.6, 2nd serum injection, 35 units. The corresponding values for Palma were 208.8, 71.7, and 164.9 units. The pepsin content fell only after the first serum injection, with Bobik, the values found being 242.2, 182.4, and 273.8 units. For Tobik, the value was less than the initial one after the second injection (54.3, 71.4, and 46.6 units). The corresponding values for Druzhok were 44.1, 58.2, and 174 units.

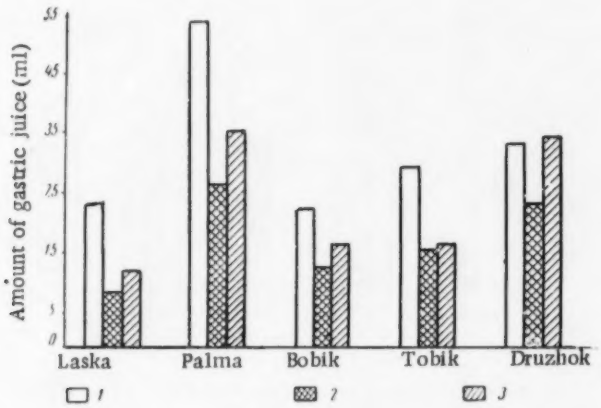


Fig. 1. Amount of gastric juice secreted in 1 hour in response to morphine and serum injections. 1) Morphine; 2) 20 ml of serum; 3) 30 ml of serum.

Our experiments show that intravenous injection of heterologous serum into healthy dogs depresses the secretory function of the gastric glands (the amount of gastric juice falls, as does its hydrochloric acid content and in many cases its enzymatic activity). This disturbance of secretory function of the stomach should, in turn, interfere with the animal's capability of digesting proteins.

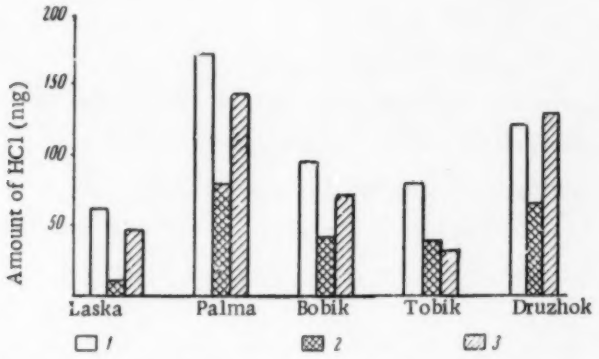


Fig. 2. Amount of hydrochloric acid secreted by dogs in 1 hour in response to morphine and serum injections. 1) Morphine; 2) 20 ml of serum; 3) 30 ml of serum.

The correctness of this supposition is confirmed by N. G. Belenky's findings [2].

He found that intravenous injection of species nonspecific serum into normal dogs in a state of nitrogenous equilibrium was always followed by a rise in the fecal nitrogen content. Considering this finding in conjunction with his earlier ones, N. G. Belenky came to the conclusion that the raised nitrogen content of the feces is ascribable to lowered assimilation of alimentary protein, but not to elimination of the species nonspecific serium proteins via the alimentary tract.

Belenky's view was latter confirmed experimentally. V. M. Rubel, in 1954 [7], working with radioactive isotopes, found that S^{35}-labeled dog plasma proteins administered to dogs were not secreted in the gastric juice.

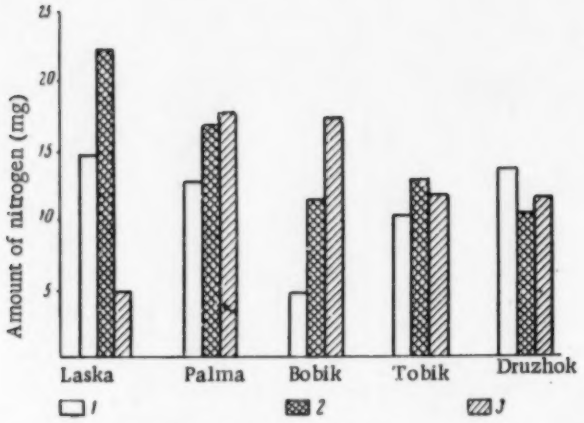


Fig. 3. Amount of nitrogen (mg) in the gastric juice of dogs, secreted over 1 hour after injections of morphine and serum. 1) Morphine; 2) 20 ml of serum; 3) 30 ml of serum.

It follows that the increased fecal nitrogen excretion found in dogs after injection of species nonspecific serum cannot be ascribed to the direct elimination of the serum proteins into the alimentary tract, but should be considered to be a consequence of depressed secretory function of the digestive glands.

To summarize our results, we conclude that the introduction of bull's serum into normal dogs causes a fall in gastric secretion, a fall in the hydrochloric acid content of the gastric juice and (in 6 of 10 experiments) in its pepsin content, and a slight rise in its nitrogen content.

LITERATURE CITED

[1] N. G. Belenky, Species Nonspecific Serum.* Moscow, 1950.

[2] N. G. Belenky, Parenteral Protein Feeding of Humans and Animals,* Moscow, 1950.

[3] E. G. Lipskaya, Proceedings of the Red Army Kuban Medical Institute,* 13, 11 (24), pp. 87-92 (1938).

[4] N. P. Pyatnitsky, Klin. Med., 1955. No. 4, 74-76.

[5] I. P. Razenkov, New Data on the Physiology and Pathology of Digestion,* pp. 282-295, Moscow, 1948.

[6] V. M. Rubel, V. I. Chukanova and S. P. Khimkin, Byull. Eksptl. Biol. i Med., 28, No. 4 (10), 275-278 (1949).

[7] V. M. Rubel, Vestnik Akad. Med. Nauk SSSR, 1954, No. 4, 13-20.

[8] A. I. Smirnov and V. F. Shirokov, Zhur. Eksptl. Biol. i Med., 4, No. 13, 694-711 (1927).

* In Russian.

EFFECTS OF CHANGES IN THE FUNCTIONAL STATE OF THE CEREBRAL CORTEX ON SECRETION OF BILE

I. T. Dzheliev

From the Laboratory for General Physiology (Director: V. N. Chernigovsky, Member Acad. Med. Sci. USSR), Institute of Normal and Pathol. Physiology, Acad. Med. Sci. USSR, Moscow

(Received December 10, 1955. Presented by V. N. Chernigovsky, Member Acad. Med. Sci USSR)

It has been shown by a number of authors that the bile-secreting function of the liver is affected by changes in the functional state of the cerebral cortex. This observation is supported by the findings of a number of workers [2, 4], showing the existence of conditioned reflex stimulation of secretion of bile. According to the findings of other authors [1, 3, 5], alterations in bile secretion may be encountered during elaboration of conditioned salivary reflexes in animals, by Pavlov's classical procedures.

In all the above researches, however, only the amount of bile secreted was measured. Insufficient work has been done on alterations in its chemical composition.

The present paper deals chiefly with the effects of alterations in the functional state of the cerebral cortex on biliary secretion of bilirubin.

EXPERIMENTAL METHODS

Our experiments were performed on three dogs with gall bladder fistulae, and with parotid gland fistulae opening to the outside. In addition, the common bile duct of one of the dogs was ligated.

The conditioned reflex activity of the dogs was studied by Pavlov's classical methods, parallel with the study of bile secretion. Before proceeding to the elaboration of conditioned reflexes in the dogs, we investigated the effect on bile secretion in two dogs of introducing hydrochloric acid into the mouth. After this, we developed a conditioned defensive reflex to acid, and followed this by differentiation to it. The conditioned reflex stimulus (the sound of a metronome in one case, and of a siren in the others) was applied during the first half-hour of each experiment.

After these experiments had been completed, we investigated the effects of perturbations of higher nervous activity. These were produced: a) by evoking "confusion," by applying a positive stimulus immediately after a negative one; b) by considerable prolongation of the time of isolated action of the conditioning stimulus; c) by changing the signal significiance of the stimuli.

In each 4-hour portion of bile collected we determined the dry content and the bilirubin content (by the van den Bergh method). In addition, we calculated the total bilirubin content of the bile and of its dry content. Fasting animals were used in all the experiments.

EXPERIMENTAL RESULTS

We could not find any sufficiently noteworthy differences between the results of the experiments in which we determined the background levels and of those in which we tested the effect of hydrochloric acid. The results showed that considerable random fluctuations in the magnitudes of the volumes of bile secreted and in its bilirubin content took place, both during the course of a single experiment, and from experiment to experiment. Thus, the bilirubin content found during an experiment varied by over 100 mg% (Figure 1). In a considerable proportion of the experiments diminution in secretion of bile coincided with increase in its bilirubin concentration, and with diminution in the absolute amount of bilirubin secreted. Only by comparing the mean values derived for each series were we able to note a certain fall in the volume of bile secreted, with a corresponding rise in its bilirubin content, for the "acid" series. Thus, in the dog Sharik, the amount of bile fell during an experiment from 30.3 to 25.5 ml, and in Glazun from 23.4 to 18.5 ml.

The process of elaboration of a conditioned reflex was associated with increased production of bile, in all the animals. In contrast to the preceding experiments, the amount of bile secreted by Kutsyi rose by an average

of 6.4 ml, by Sharik by 8.4 ml, and by Glazun by 10 ml. There was at the same time a pronounced fall in the concentration of bilirubin in the bile. The most significant result of this series of experiments is, in our view, the considerable change in the course of secretion of bile. There was a marked fall in the amplitude of the variations in the volume of bile secreted and in its bilirubin content, and this applied not only to a given experiment, but also to the whole series. The effect noted was thus of the nature of a stabilization of biliary secretion (Figure 2).

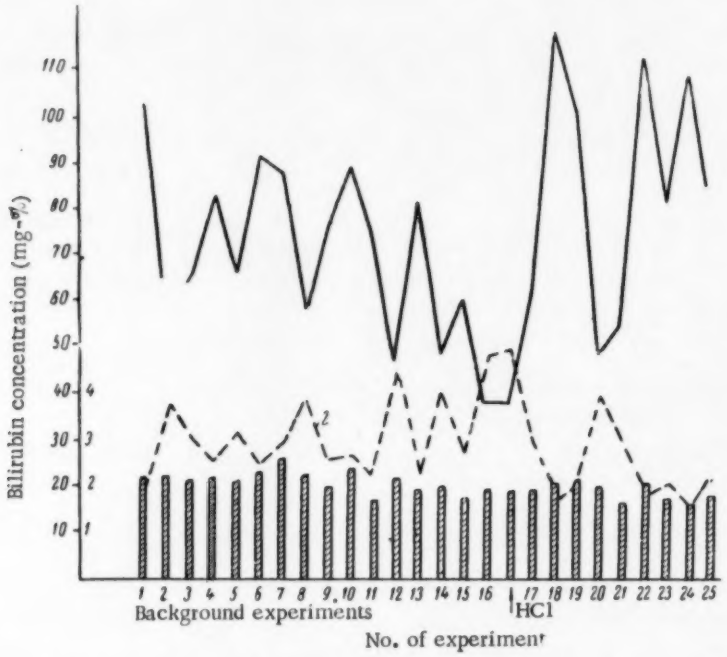


Fig. 1. Absolute amount of bilirubin, and its concentration in bile secreted by the dog Sharik (background experiments, and experiments on the action of hydrochloric acid by itself). 1) Concentration of bilirubin; 2) volume of bile secreted during the experiment; vertical columns: amount of bilirubin, in mg for each experiment.

We consider the increase in the amount of bile secreted, as well as the alteration in the nature of the process, to be results of the formation of a stable temporal link in the cerebral cortex. This view is supported by the observation that these changes in the magnitude and the nature of the biliary secretion become evident only after the given reflex has become firmly established. Moreover, the stabilization of bile secretion persists only as long as the conditioned stimuli are systematically applied. Interruption of the experiments was followed by violent and disorderly fluctuations in the volume of bile secreted, and in its bilirubin concentration, such as were observed in the "background" experiments.

The elaboration and establishment of differentiation of already existing conditioned reflexes had no perceptible effect on secretion of bile. Only during the first days of application of differentiating stimuli did we observe any more pronounced fluctuations in the volume of bile secreted, and in its bilirubin concentration. As the differentiations became more firmly established these fluctuations became much smaller, so that the "stabilization" of the bile-secreting process found in the previous series, during establishment of the conditioned reflexes, was even more marked in this series of experiments. Apart from this, the establishment of differentiation in our animals was associated with a fall in the amount of bile secreted (the average fall amounted to 0.5 ml for Kutsyi, and to 7.3 ml for Glazun), and with a certain increase in the bilirubin concentration of the bile.

Derangements of higher nervous activity, which were observed in all the animals subjected to the treatments described above, caused well-defined alterations in bile secretion. The stabilization in the amount of bile secreted and in its bilirubin concentration, which were characteristic of the phases of elaboration and firm establishment of the conditioned reflexes, and of their differentiation, disappeared. Chaotic fluctuations in the

amount secreted reappeared, both in a single experiment, and from experiment to experiment. The bilirubin content of the bile suffered a similar disturbance. These alterations in the nature of the secretion of bile proceeded against a background of general, although slight, diminution in the amount of bile secreted.

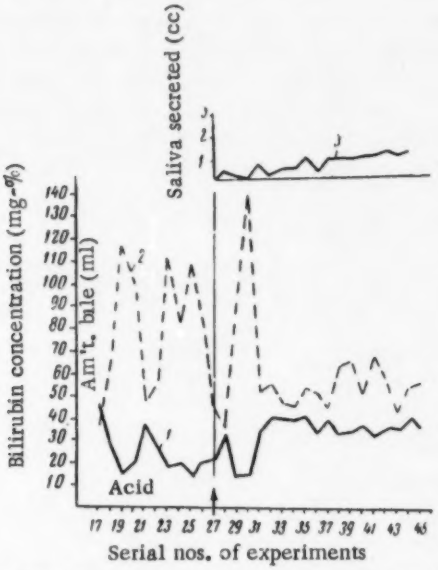


Fig. 2. Amount of bile secreted in each experiment by the dog Sharik, and its bilirubin concentration, after establishment of a conditioned reflex (beginning with the 27th experiment). 1) Amount of bile secreted, in ml per experiment; 2) its bilirubin concentration, in mg %; 3) amount of conditioned reflex salivation, in cm of the manometer scale.

We may conclude from our experiments that the variations in the functional state of the cerebral cortex which were present were of sufficient magnitude to bring about changes in the nature of bile secretion, and in its bilirubin concentration. We may suppose these changes to be caused by induction processes which arise in the cerebral cortex during the formation of the conditioned reflexes or of their differentiation.

In distinction to the amount of bile and to its bilirubin concentration, we could find no significant variations in the absolute amounts of bilirubin secreted. The variations in these values, from experiment to experiment over the whole period of the research, were of an undulant nature, and were inconsiderable. It is evident that they are not related to our interference with cortical processes (Fig. 3).

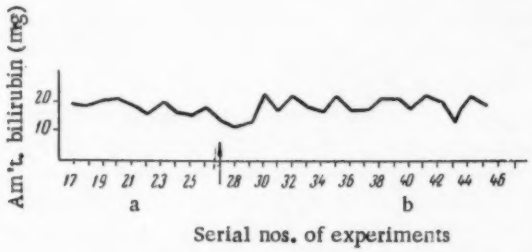


Fig. 3. Absolute amounts of bilirubin secreted with the bile of the dog Sharik.
a) In background experiments; b) during formation of a conditioned reflex.

The relatively small range of variations in the absolute amount of bilirubin secreted with the bile may be explained as being due to its being a degradation product of hemoglobin, a relatively constant level of which is essential for the proper functioning of the organism. It is known that a certain parallellism exists between the amount of hemoglobin in the blood and the amount of bilirubin secreted with the bile [6]. This relative constancy is apparently a consequence of the role of bilirubin in hemopoiesis, since it has been found that one of the conditions for the proper functioning of the hemopoietic process is the normal secretion of bilirubin into the intestine [7]. It is evident that the process of formation of the bile pigments depends on complex biochemical processes, which proceed continually, chiefly in the liver. It may be thought that the changes in the functional state of the cerebral cortex produced in our experiments were of insufficient magnitude to influence the formation of bile pigments. Were such relatively slight changes in cortical function as are associated with the elaboration of a conditioned reflex, or with its differentiation, to be able significantly to affect the course of complex metabolic processes in the organism, we would expect there to be continual and violent fluctuations in the composition of the internal medium of the organism. We know, however, that organisms maintain the constancy of their internal medium with great precision.

LITERATURE CITED

[1] S. L. Balakin, Fiziol. Zhur. SSSR, 29, No. 6, 505-510 (1940).

[2] E. P. Ivanov, Fiziol. Zhur. SSSR, 13, No. 2, 281-285 (1930).

[3] V. G. Prokopenko, The Cerebral Cortex, and the Secretory Function of the Liver,* Thesis, 1939.

[4] A. V. Rikkl, Fiziol. Zhur. SSSR, 13, No. 2, 268-280 (1930).

[5] M. G. Shmulevich, Fiziol. Zhur. SSSR, 25, No. 6, 919-924 (1938).

[6] A. Sonnefeld, Med. Klin., 1922, N. 44, S. 1407-1409.

[7] A. Zih, Ztschr. f. d. ges. exper. Med., 1939, Bd. 104, S. 672-678.

BILE SECRETION AFTER PARTIAL RESECTION OF THE LIVER OF DOGS WITH AN EXTERIORIZED COMMON BILE DUCT

B. P Solopaev

From the Growth and Development Laboratory (Director: the late Prof. M. A. Vorontsova), Institute of Experiment Biology (Director: Prof. I. N. Maisky), Acad. Med. Sci. USSR

(Received January 9, 1956. Presented by V. N. Chernigovsky Member Acad. Med. Sci. USSR)

Structural changes taking place in the liver during the process of its regeneration following traumatic injury have been studied in a number of mammalian species [3, 5, 6, 8, 9, 10]. During reparative regeneration of the mammalian liver the resected portion of the organ is not restored, although the final weight and size of the liver are the same as initially, as a result of intensive proliferation of the surviving tissue. This mode of regeneration has been termed regenerative hypertrophy by M. A Vorontsova [1].

While the histogenesis of hepatic regeneration has been the subject of numerous researches, only a very few papers have been devoted to the study of alterations in the functional activity of the liver after its traumatization and during the subsequent regenerative process [7]. This problem is, however, of considerable current interest, in particular for surgical practice [2, 4]. A study of the functional changes parallel with the morphological ones encountered during regeneration of the liver would permit of a correlated morphophysiological treatment of the process.

We investigated the alterations in the bile secreting function of the liver during regeneration following traumatic injury. The experiments were performed on 1-2 year-old dogs.

* In Russian.

EXPERIMENTAL METHODS AND RESULTS

After exteriorizing the common bile duct, by I. P. Pavlov's method, we determined the normal secretion of bile in response to meat, recording the amount secreted in 15 minute intervals, its color, opacity, and bilirubin and cholesterol content. Partial resection of the liver was then performed, after which bile secretion again followed.

After exteriorization of the common bile duct the dog Dik produced from 4.5 to 18.7 cc of bile per hour, mean secretion 10 ± 0.83 cc. Part of the right anterior lobe of the liver was then removed. Secretion of bile began on the third day after the operation, as soon as the dog was put into the stand.

The first portion of bile was of a milky-yellow color, and the succeeding ones were of the ordinary color. The rate of secretion curve was level, without any abrupt fluctuations such as are typical of the normal curve. On the 6th day there was no latent period of bile secretion, and the bile was of the ordinary color; the 1 hour secretion was 19.5 cc. During the succeeding days, the amount of bile secreted diminished, gradually approaching the normal value.

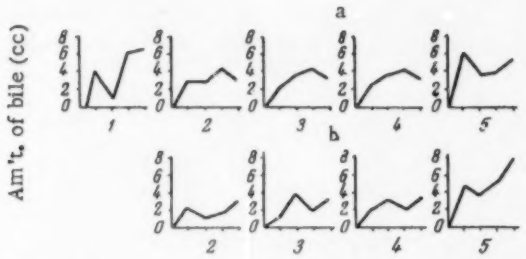


Fig. 1. Bile secretion in response to meat, after partial hepatic resection in the dog Borok. a) After resection of part of the right anterior lobe of the liver; b) after repeated resection of the right anterior lobe. 1) Before operation; 2) one day after operation; 3) 2 days after operation; 4) 3 days after; 5) 4 days after operation. Abscissa: time, in 15 minute intervals.

After exteriorization of the common bile duct the dog Borok produced 8.8 to 26 cc of bile per hour, a mean value of 16.2 ± 1 cc; the bilirubin content varied from 20 to 200 mg%, mean value 65 ± 20 mg%, and the cholesterol content was 80-230 mg %.

Part of the right anterior lobe of the liver was removed from this dog. During the first few days after the operation no latent period for secretion of bile could be observed. Approximately equal amounts were secreted at 15 minute intervals, so that the rate of secretion curve was horizontal (Figure 1, a). Five days after the operation the amount of bile secreted during 1 hour was 21.5 cc. The amount secreted had gradually returned to the normal value by the end of the second week. The bilirubin content was somewhat elevated after the operation (220 mg%). The cholesterol content was within normal limits.

The remainder of the right anterior lobe of the liver was removed from the dog Borok on the 44th day after the first operation;

A second operation, involving removal of the remainder of the right anterior lobe and of a portion of the left posterior lobe weighing 80 g, was performed on the dog Dik, 4 months after the first operation. The effects on bile secretion were similar to those seen after the first operation.

There were differences in the rate of secretion curves, for 15 minute intervals, which were not so level as after the first operation, and showed fluctuations which were not, however, as marked as for normal bile secretion (Figure 1, b). Apart from this, the normal pattern of bile secretion was reestablished much sooner than after the first operation (towards the end of the first week).

Similar changes were seen in the bilirubin and cholesterol contents of the bile.

After exteriorization of the common bile duct, the dog Belka secreted from 2.2 to 9.5 cc of bile per hour, mean secretion 6.3 ± 1.2 cc.

A portion of the left hepatic lobe weighing 32 g was resected (Figure 2, a). On the following day, in response to 200 g of meat, the bile secreted differed in successive portions: the 1st portion (0.8 cc) was watery and faintly colored, the 2nd (2.6 cc) was turbid and of a dark green color, and the 3rd (4.7 cc) separated into an upper, yellow layer and a lower, dark green layer, the 4th (0.4 cc) was turbid and greenish-white, and the 5th (0.5 cc) was stratified.

After another day, a great amount of watery, whitish fluid (white bile) began to flow from the duct, as soon as the dog was placed in the stand; 70 cc were secreted in 15 minutes, and 181 cc after an hour.

The fluid contained considerable amounts of greyish-white, flocculent precipitate. Microscopic examination of the deposit showed the presence of cell detritus, well-preserved sheets of common bile duct epithelium, and cholesterol crystals. The amount of deposit was greatest during the 2nd hour from the beginning of feeding.

After 2 days, feeding was immediately followed by secretion of white bile, in smaller amount than on the previous day, although still 10 times greater than the normal value; 81 cc of white bile were secreted over an hour. On this day three strata were visible in the bile: an upper, pale yellow layer, a middle, almost colorless one, and a lower, greyish-white one. Traces of bilirubin and cholesterol were found in the white bile.

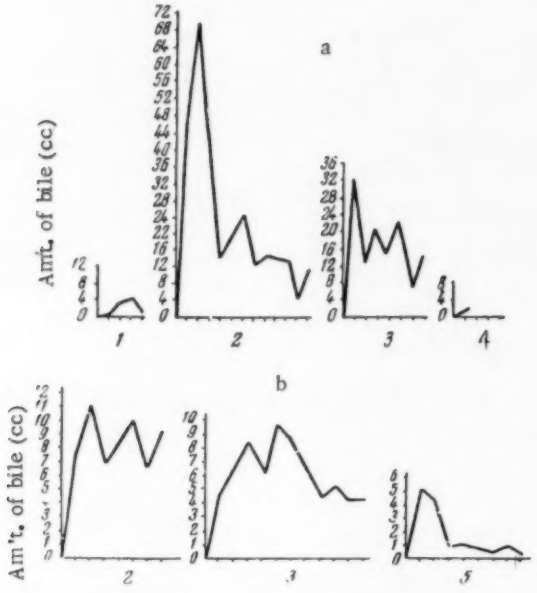


Fig. 2. Secretion of bile in response to meat after partial resection of the left hepatic lobe. a) Belka; b) Belyi. 1) On the day of operation; 2) 1 day after; 3) 2 days after; 4) 3 days after; 5) 4 days after the operation. Time scale as for Figure 1.

Nitrogen was determined in two portions of bile (with the least and the greatest amount of deposit), by the Kjeldahl method. We found 0.0196 mg/ml of nitrogen in the portion containing less sediment, and 0.02 mg/ml in that containing more sediment.

After 4 days the dog was feeble, and lay down most of the time, being reluctant to stand up. It refused meat when placed in the stand, but drank some glucose water. Bile at once started to flow, being transparent, and of

a brownish-green color; 2.5 cc were secreted during 15 minutes. At the 18th minute the dog collapsed in the harness, and the experiment was abandoned. It died in acute dehydration, despite all measures taken to save it.

At autopsy we found acute dehydration of the tissues, with atrophy of the muscles. The bile ducts were patent, and viscous, dark green bile could be expressed from the gall-bladder. Microscopic examination of stained sections of the liver showed marked dilatation of the pericapillary spaces and of the capillaries, a disordering of the hepatic trabeculae, and degenerative changes in the liver cells.

After exteriorization of the common bile duct, the dog Belyi secreted from 5.9 to 8.6 cc of bile per hour, mean secretion 7.0 ± 0.5 cc, containing bilirubin 48-249 mg %, mean value 100±61 mg %, and cholesterol 80-869 mg %, mean value 137±47 mg %.

One after resection of a portion of the left anterior lobe of the liver, weighing 24 g, the dog began to discharge white bile, in the form of a turbid greyish-white fluid with a large amount of sediment. The volume of white bile was 4 times greater than the amount of bile usually secreted by this animal (Figure 2, b). Smears of the bile showed various cells, in a more or less advanced stage of degeneration, isolated cholesterol crystals, and microorganisms. The first portions of white bile contained less sediment, and the nitrogen content amounted to 6.95 mg/ml, corresponding to 43.48 mg/ml of protein; the nitrogen content of portions of bile giving a more copious sediment was 8.54 mg/ml, corresponding to 53.4 mg/ml of protein.

Two days after the operation, as soon as the dog was placed in the stand and given food, it began to discharge white bile, in slightly lesser amount than on the previous day, although still considerably more than before the operation. The bile collected on this day separated into 3 layers, as for the dog Belka.

After 4 days, ordinary bile was being discharged, being dark brown in the first portion, and light brown in the succeeding ones. On the following days the amount of bile secreted fell steadily, falling to zero on the 10th day. Flow of bile was renewed after 5 days, and became similar to that before the operation.

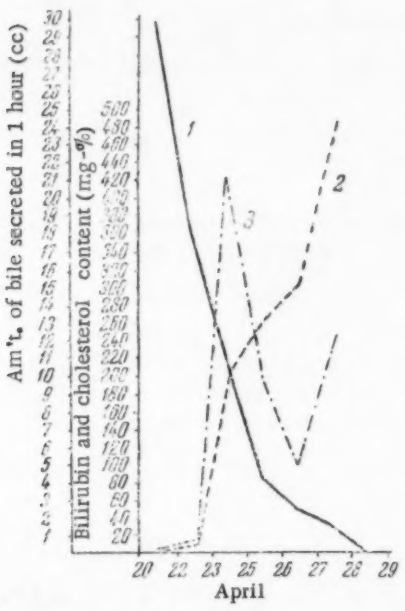


Fig. 3. Relation of the amount of bile secreted over 1 hour to its bilirubin and cholesterol contents, after partial resection of the left hepatic lobe of the dog Belyi, on April 20. 1) Amount of bile; 2) bilirubin content; 3) cholesterol content.

During the first 2 days the bilirubin content of the bile was very small (traces only). Thereafter, parallel with diminution in the volume of bile discharged, its bilirubin content rose to values considerably exceeding the normal ones (Figure 3). The cholesterol content was very small (traces) during the first two days, and was in the normal range thereafter (Figure 3).

DISCUSSION OF RESULTS

The disturbances in secretion of bile following partial resection of the liver may be considered in two groups: minor changes seen after removal of part of the right lobe, and after repetition of this operation, and major changes, manifested by discharge of white bile, following partial resection of the left hepatic lobe.

Our findings are evidence of the extraordinarily large compensatory capabilities of the liver, which is able, within a very short time after infliction of very considerable trauma, to restore its impaired functions, in particular that of bile secretion.

Different results were obtained for injury of the right and the left lobe, and this is probably due to their differences in blood supply. It may hence be concluded that, in operations involving surgery of the liver, not only the amount of tissue removed, but also its location, should be taken into consideration.

Disturbances of bile production are much less severe after repetition of the resection, and restitution of normal biliary function takes place much sooner.

LITERATURE CITED

[1] M. A. Vorontsova, Regeneration of Lost Organs in Animals and Man,* Moscow, 1953.

[2] A. Ya. Galushko, Resection of the Liver, in Experimental and Clinical Practice,* Thesis, Gorky, 1948.

[3] N. I. Grigorev, Transactions of the V All-Union Congress of Anatomists, Histologists, and Embryologists,* Leningrad, 1951.

[4] I. V. Danilov, Khirurgiya, 1939, No. 4.

[5] R. P. Zhenevskaya, Trudy Inst. Morfol. Zhivotn., 1954.

[6] V. A. Meister, Regeneration of Hepatic Tissue after removal of Entire Lobes of the Liver, up to Four-Fifths its Total Mass;* Kiev, 1894.

[7] Ibid, Biological-Chemical Studies on Hepatic Tissue;* Kiev, 1894.

[8] W. Podwyssozki, Beitr. Pathol. Anat. u. allgem. Fathol.

[9] F. A. Fishback, Arch., path., 1929, 7.

[10] E. Ponfick, Virchow's Arch., 1887, Bd. 118; E. Ponfick, Virchow's Arch., 1890, Bd. 119.

EFFECT OF THE DRUG TETAMON-I ON THE BILE SECRETING FUNCTION OF THE LIVER

N. P. Skakun and A. Ya. Sereda

From the Chair of Pharmacology (Director: Assistant Prof. Yu. A. Petrovsky), Lvov State Medical Institute (Director: L. N. Kuzmenko)

(Received December 12, 1955. Presented by V. N. Chernigovsky, Member Acad. Med. Sci. USSR)

We showed in our previous communication [10] that Difenin, which hinders transmission of nerve impulses in N-cholinergic systems, exerts a marked inhibitory effect on the biliary function of the liver.

The present paper deals with the nature of the effect of Tetamon-I (tetraethylammonium iodide) on bile formation in the liver.

As has been shown by the work of K. M. Bykov, A. V. Rikkl, E. P. Ivanov, and S. L. Balakin [4, 5, 7, 9] and others, bile secretion is under the control of the central nervous system, and disturbances of the nervous connections between the central nervous system and the liver should lead to alterations in hepatic function.

Tetraethylammonium derivatives inhibit transmission of impulses in N-cholinergic systems of peripheral ganglions, chiefly of the parasympathetic system [1, 2, 3, 6, 8, 11].

EXPERIMENTAL METHODS

The experiments were performed on 4 dogs provided with permanent gall-bladder fistulae, and with ligated common bile ducts. Each experiment lasted 4 hours, and we determined the amount of bile secreted, and its bile acid content, for each hour. In the first series of experiments we examined the effect of Tetamon-I on spontaneous bile secretion, and in the second series we examined the effect during digestion. The injections of the drug were followed immediately by breakfast, consisting of 250 ml of milk and one large hen's egg. Tetamon-I was injected intravenously (in some experiments intramuscularly), in doses of 0.5-2 ml, corresponding to

---

*In Russian.

3.3-14 mg per kg body weight. In all, we performed 44 experiments.

TABLE 1

Effect of Tetamon-I on Flow of Bile in Dogs

Dog	Amount of bile, in ml										
	before injection of drug					after injection of drug					
	1 hr.	2 hrs.	3 hrs.	4 hrs.	total for 4 hrs.	dose in mg/kg	1 hr.	2 hrs.	3 hrs.	4 hrs.	total for 4 hrs.
a) For spontaneous flow of bile											
Mukha	7.3	8.7	10.3	9.5	35.8	7.0	6.9	4.9	11.2	8.9	31.9
	7.3	8.7	10.3	9.5	35.8	14.0	4.4	6.4	11.2	8.9	30.9
Alfa	9.3	9.2	9.5	10 3	38.3	6.3	6.5	5.7	5.8	7.3	25.3
Kashtanka	12.1	12.1	10.7	7.5	42.4	3.3	13.8	10.6	7.8	8.0	40.2
	12.1	12.1	10.7	7.5	42.4	6.6	7.8	6.9	7.4	7.3	29.4
b) For flow of bile during digestion											
Sultan	6.5	6.9	8.0	10.0	31.4	5.0	2.7	6.0	9.0	7.3	25.0
Mukha	6.5	9.3	7.9	12.2	35.9	7.0	3.5	6.6	10.0	11.2	31.3

EXPERIMENTAL RESULTS

As is evident from Table 1, administration of Tetamon-I in most cases caused a reduction in the spontaneous flow of bile. The amount of bile produced by the dog Mukha after intravenous injection of 1 ml of the drug (7 mg per kg body weight) fell by about 11% over 4 hours, and after 2 ml of the drug by 13.7%. The effects were more marked in the dogs Alfa (diminution in flow of 34% after a dose of 6.3 mg per kg body weight), and Kashtanka, in which a dose of 6.6 mg per kg body weight lowered bile secretion by 30.7%. No changes were seen in the dog Kashtanka after injection of half this dose (3.3 mg per kg body weight). Inhibition of bile secretion took place chiefly during the first two hours after injection, with subsequent rise to the normal level.

At the same time, we observed changes in the composition of the bile, in particular, a regular fall in the bile acids content, which was evident over the entire 4 hours of observation (Table 2).

Determination of the total bile acid content showed that this had diminshed by 30-40% for spontaneous flow of bile. Depression of bile acid secretion was particularly evident during the first two hours of action of the drug.

Similar results were obtained in experiments on the effect of Tetamon-I on bile secretion during digestion. The flow fell by 12.5-20.4%, for the dogs Sultan and Mukha, and the bile became much more dilute, with a fall in the content of bile acid salts of 22.5-42%.

It thus appears that the effect of tetraethylammonium iodide (Tetamon-I) is to lower the amount of bile produced, and to reduce the concentration of its chief component, the bile acids. This is evidence of depression of the biliary function of the liver, inasmuch as synthesis of bile acids is a specific function of the liver.

It can only be assumed that this effect is due to the hindrance of transmission of nerve impulses in N-cholinergic systems, mostly of the parasympathetic ganglions, by tetraethylammonium salts.

TABLE 2

Effect of Tetamon-I on the Bile Acid Content of the Bile

Dog	Content of bile acid salts in the bile, in mg-%										
	before injection of drug					after injection of drug					
	1 hr.	2 hrs.	3 hrs.	4 hrs.	total for 4 hrs.	dose in mg/kg	1 hr.	2 hrs.	3 hrs.	4 hrs.	total for 4 hrs.
a) For spontaneous flow of bile											
Mukha	3 280	3 460	2 840	3 210	1 137.9	7.0	2 670	2 620	2 330	2 080	791.2
	3 280	3 460	2 840	3 210	1 137.9	14.0	3 000	2 080	2 620	2 540	784.6
Alfa	1 920	2 000	1 920	1 910	741.7	6.3	1 990	1 820	1 580	1 670	446.0
b) For flow of bile during digestion											
Sultan	4 880	4 840	4 620	4 440	1 464.8	5.0	4 230	3 930	2 960	3 210	850.7
Mukha	2 850	2 430	2 300	2 310	874.8	7.0	2 540	2 340	2 180	1 930	677.5

LITERATURE CITED

[1] S. V. Anichkov, Novosty Med., No. 12, pp. 1-11, Moscow, 1949.

[2] S. V. Anichkov, Problems of the Pharmacology of the Vegetative Nervous System,* pp. 5-19, Moscow, 1952.

[3] S. V. Anichkov, In the book: Pharmacology of New Drugs,* pp. 5-13. Leningrad, 1953.

[4] S. L. Balakin, Fiziol. Zhur. SSSR, 29, Issue 6, No. 12, 505-510 (1940).

[5] K. M. Bykov, The Cerebral Cortex and the Viscera,* Moscow-Leningrad, 1947.

[6] Z. I. Vedeneeva, in the book: Pharmacology of New Drugs,* pp. 44-50. Leningrad, 1953.

[7] E. P. Ivanov, Fiziol. Zhur. SSSR, 13, No. 2, 281-285 (1930).

[8] T. A. Melnikova, in the book: Pharmacology of New Drugs,* pp. 104-107. Leningrad, 1953.

[9] A. V. Rikkl , Fiziol. Zhur. SSSR, 13, No. 2, 268-280 (1930).

[10] N. P. Skakun, Byull. Eksptl. Biol. i Med., 38, No. 11, 51-52 (1954).

[11] M. L. Tarakhovsky, Farmakol. i Toksikol., 15, No. 2, 43-48 (1952).

* In Russian.

PANCREATIC SECRETION IN RESPONSE TO DIFFERENT AMOUNTS AND CONCENTRATIONS OF HYDROCHLORIC ACID SOLUTIONS*

O. B. Sobieva

From the Chair of Human and Animal Anatomy and Physiology (Director: Prof. V. E. Robinson), Ryazan Pedagogic Institute

(Received November 28, 1955. Presented by P. S. Kupalov, Member Acad. Med. Sci. USSR)

The study of the use of hydrochloric acid for stimulating the external secretion of the pancreas is of considerable theoretical and practical importance.

It has been shown by the work of I. P. Pavlov and his school that one of the most powerful stimuli of pancreatic secretion is the action of acids, in particular hydrochloric acid, on the duodenal wall [1, 2, 3, 4].

Although these workers established the important fact of the specific stimulatory action of hydrochloric acid, they did not undertake a study of the quantitative aspects of this action. They did not investigate the effect on pancreatic secretion of introducing into the duodenum hydrochloric acid solutions of concentrations higher than the physiological ones.

I. L. Dolinsky expressed the view, in 1894, that introduction of more concentrated solutions of hydrochloric acid into the duodenum should cause a greater secretory response than if 0.5% hydrochloric acid is used [2].

Apart from this, we could find no references in the literature to the effect of varying the amount of hydrochloric acid introduced on pancreatic secretion.

Nor has the secretory response of the pancreas to introduction into the duodenum of acid together with pancreatic secretion been studied, although this procedure more closely approaches the conditions applying during natural digestion.

EXPERIMENTAL METHODS

The experiments were performed on two dogs (Palma and Tarzan), provided with a pancreatic fistula, by our modified method [5]. In addition, the dogs had a gastric fistula, which remained open during the whole time of the experiment.

Hydrochloric acid of different concentrations was introduced from a buret during 1 minute, at 15 minute intervals.

The amount of pancreatic secretion was measured at 15 minute intervals, and from these values we derived the values per hour, and for the whole experiment, lasting two hours.

In some experiments, the pancreatic secretion was reintroduced into the duodenum.

In all, we performed 106 experiments on both dogs.

EXPERIMENTAL RESULTS

We first determined the background values, for periodic secretion and for secretion following introduction of water. We found that periodic secretion took place in both dogs.

Introduction of water into the duodenum caused a certain rise in pancreatic secretion, as compared with spontaneous periodic secretion.

We then examined the effect on pancreatic secretion of varying the concentration and the amount of acid introduced into the duodenum

* Read at the Pavlov Centenary Meeting of the Academies of Sciences and Medical Sciences, 1949.

The results are presented in Figure 1, from which it appears that introduction of 25 ml of 0.05% hydrochloric acid caused a stimulation of pancreatic secretion, as compared with the background values.

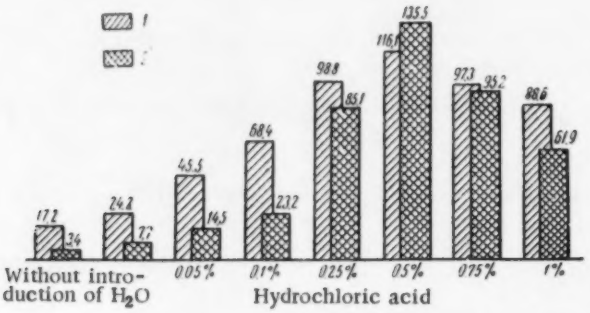


Fig. 1. Amount of pancreatic secretion produced in response to introduction into the duodenum of 25 ml of hydrochloric acid of various concentrations. 1) The dog Palma; 2) the dog Tarzan.

Introduction of 25 ml of 0.05 to 0.5% hydrochloric acid caused a rise in pancreatic secretion in both dogs, which, however, showed individual variations: the increase in secretion varied parallel with the concentration of acid for Palma, whereas Tarzan showed only a weak response to low concentrations, with a sudden rise when the hydrochloric acid concentration reached 0.25%.

The secretory response to one and the same concentration of hydrochloric acid varied from day to day, for both dogs, which is doubtless evidence of the profound influence of the state of the higher nervous system of the animals on pancreatic secretion.

Raising the acid concentration to 0.75% caused a marked fall in pancreatic secretion, in both dogs, and the effect of 1% acid was still more pronounced. For Palma it amounted to 70% of the amount secreted in response to 0.5% acid. The fall in secretion in response to 1% acid was particularly evident in the experiments with Tarzan, only 45% of the amount given by 0.5% acid being produced.

Thus, our experiments do not confirm Dolinsky's supposition that still further increase in pancreatic secretion can be achieved using relatively large volumes (25 ml) of hydrochloric acid solutions of concentrations exceeding the "physiological" range.

The results of the experiments on the effect of varying the amounts of acid introduced on pancreatic secretion are presented in Figure 2.

Only a very slight rise in the amount of pancreatic secretion results from introduction into the duodenum of 5 ml of 0.05% hydrochloric acid; the effect is about the same, or even in some cases smaller, than when the same amount of water is given.

Increasing the amount of 0.05% acid to 15 ml had a slightly greater effect, but even this barely exceeded that given by water alone.

The response to introduction of 25 ml of 0.05% acid was twice as great as that to an equal volume of water.

Increasing the amounts of 0.25% and 0.5% hydrochloric acid caused a corresponding increase in the amount of pancreatic secretion produced.

Introduction of 5 and 15 ml of 1% acid produced a greater amount of secretion than did the same volumes of any other concentration of acid. Raising the volume of 1% acid to 25 ml caused a marked fall in pancreatic secretion.

It thus appears that only large amounts (25 ml) of the more concentrated acid exert a pronounced inhibitory effect on pancreatic secretion.

The effects of introducing different solutions of hydrochloric acid directly into the duodenum or via the stomach are presented in Fig. 3.

Introduction through the stomach of 25 ml of 0.05 and 0.25% hydrochloric acid gave a slightly smaller effect than when the acid solutions were introduced directly into the duodenum. Practically the same secretory response was found when 25 ml of 0.5% acid was introduced via the stomach or directly into the duodenum.

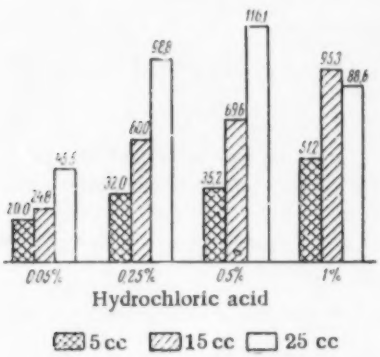


Fig. 2. Production of pancreatic secretion in response to introduction into the duodenum of various amounts of hydrochloric acid of different concentrations.

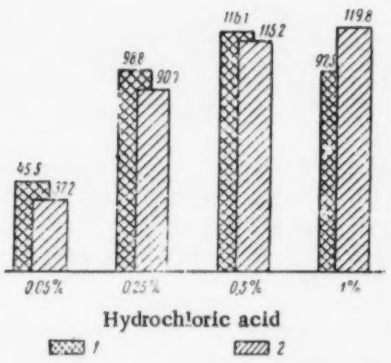


Fig. 3. Production of pancreatic secretion in response to introduction of different solutions of hydrochloric acid (1) into the duodenum, and (2) into the stomach.

The production of pancreatic secretion after introduction of 25 ml of 1% hydrochloric acid via the stomach was greater than when it was placed directly into the duodenum.

An explanation of this finding is that 1% hydrochloric acid, being a powerful irritant, evokes a defense reaction of the gastric wall, involving copious secretion of mucus. As as result, some of the acid is neutralized by the alkaline gastric mucus secretion. The concentration of the acid then approaches more closely the optimum values, as a result of which pancreatic secretion exceeds that found when the acid is placed directly into the duodenum.

We also studied the effect on pancreatic secretion of introducing 25 ml of 0.5 and 0.75% hydrochloric acid into the duodenum, simultaneously with pancreatic juice. Under these conditions, and using 0.5% hydrochloric acid, the amount of pancreatic secretion fell by 20% below that found when pancreatic juice was not reintroduced into the duodenum.

This effect may be explained as being due to partial neutralization of the 0.5% acid by alkalis present in the pancreatic juice, thus lowering the stimulatory power of the acid solution. For the same reason, the amount of pancreatic secretion produced in response to introduction of 0.75% acid together with pancreatic juice was 12% greater than with acid alone. In this case, neutralization of part of the acid raised the stimulatory power of the solution.

LITERATURE CITED

[1] A. A. Valter, Secretory Function of the Pancreas.* Thesis, St. Petersurg, 1897.

[2] I. L. Dolinsky, Effect of Acids on Pancreatic Secretion,* Thesis, St. Petersburg, 1894.

[3] A. R. Krever, Analysis of the Secretory Function of the Pancreas,* Thesis, St. Petersburg, 1899.

[4] L. P. Popelsky , Russk. Vrach, 1902, No. 49, 1797-1799.

[5] O. B. Sobieva and V. E. Robinson, Fiziol. Zhur. SSSR, 39, 629-631 (1953).

* In Russian.

SECRETION OF INTESTINAL ENZYMES BY ISOLATED INTESTINAL SEGMENTS AT DIFFERENT INTERVALS AFTER "DENERVATION"

L. S. Fomina

From the Laboratory for the Physiology of Digestion (Director: Prof. G. K. Shlygin), Institute of Nutrition (Director: Prof. O. P. Molchanova, Corresponding Member Acad. Med. Sci. USSR), Acad. Med. Sci. USSR, Moscow

(Received December 6, 1955. Presented by V. N. Chernigovsky, Member Acad. Med. Sci. USSR)

Much work has been done on the effect on intestinal secretion of severing various nerve trunks [1, 5, 6, 8, 16, 18]. There are indications [15] that the paralytic secretion found after severing nervous connections with the intestine takes place during their degeneration. Paralytic secretion ceases between the 3rd and the 4th week after section of the nerves, and this coincides with the time required for the growth of new nerves, together with the blood vessels.

Most of the authors who have studied paralytic secretion have taken into consideration only the fluid part of the intestinal secretion, with its dissolved enzymes, but have not paid sufficient attention to the solid fraction of the secretion. Very few papers have appeared dealing with the intestinal secretion as a whole.

The papers published from the Institute of Nutrition of the Academy of Medical Sciences, USSR, of recent years [9, 10, 13] have shown the necessity, in studying the enzyme-secreting function of the intestine, of including in the survey the solid fraction of the secretion, with its contained enzymes.

Observation of "denervated" intestinal segments has usually been conducted over a relatively short period of time after "denervation," and the effect of new ingrowth of nerves has not been considered.

There are indications [4, 2] of partial restoration of function of "denervated" organs during ingrowth into them of nerves derived from other systems. It may hence be expected that the ingrowth of nerves into "denervated" intestinal segments should have some effect on their functioning.

We have in this research attempted to ascertain the effect of "denervation" of isolated intestinal segments on the secretion of enzymes, in both the fluid and the solid fractions of the secretion, and to follow the changes in secretory function of the isolated segment over greater periods of time after "denervation.". The term "denervation" is written in quotation marks because the intramural nervous system of the isolated intestinal segment remains intact.

EXPERIMENTAL METHODS

The experiments were performed on 12 dogs, each of which had two isolated intestinal segments, formed from neighboring parts of the intestine. One of these was isolated by the Thiry procedure, and the other was "denervated" in two ways: in 6 dogs the segment of intestine was implanted under the skin of the abdomen, and 1-5 months later we severed its neurovascular bundle intraabdominally. The other way, practiced on 6 dogs, consisted of cutting all visible branches of the mesenteric nerves, and painting the vessels with 5% phenol solution, leaving the isolated segment within the peritoneal cavity, with the blind end, similarly to the Thiry preparation, fixed to the lesser omentum, so as to put them in a convenient position for the collection of secretion. In 8 dogs the isolated segments were from the upper regions of the intestine, and in 4 dogs from the lower ones. The secretion from the isolated segments was collected over 5-hour periods from dogs which had been fasted for 18 hours. During the first 2 months after "denervation" the experiments were performed 2-3 times a week, after which they were repeated in series of 6-8 every 3-6 months.

We recorded the amount of the solid and fluid fractions of the secretion, after which it was homogenized, and, using the quantitative methods applied in our other researches, we determined its content of enterokinase [14, 7], alkaline phosphatase [12], invertase, peptidase, and lipase [10]. The enzyme contents were calculated per g of solid content of the juice, and the amount of enzymes secreted per hour was also calculated.

We observed an abrupt change in the secretory function immediately after transection of the neurovascular bundle supplying the transplanted segment of intestine; paralytic secretion began. The amount of secretion produced in unit time (Figure 1) rose markedly, reaching a maximum on the 5-7th day, and there was a considerable admixture of blood. The amount of secretion then fell abruptly, to a value lower than the inital one, and it was no longer tinged with blood. The amount of solid fraction of the secretion also rose during the phase of paralytic secretion, and then fell abruptly, in the same way as the liquid phase.

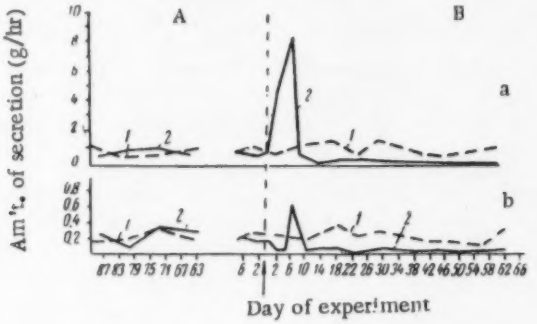


Fig. 1. Amount of secretion produced by isolated intestinal segments. A)Before severing the neurovascular bundle; B) after severing it; a) total amount of secretion; b) amount of solid fraction of secretion; 1) Thiry preparation; 2) transplanted segment. The arrow indicates when the neurovascular bundle supplying the abdominal transplant was cut.

Secretion of all the enzymes fell very considerably during the phase of paralytic secretion (Figure 2). The content of some of the enzymes fell almost to zero immediately after "denervation;" thus, enterokinase could not be detected on the 2nd and 3rd days, either in the homogenate or in the solid secretion. The content of

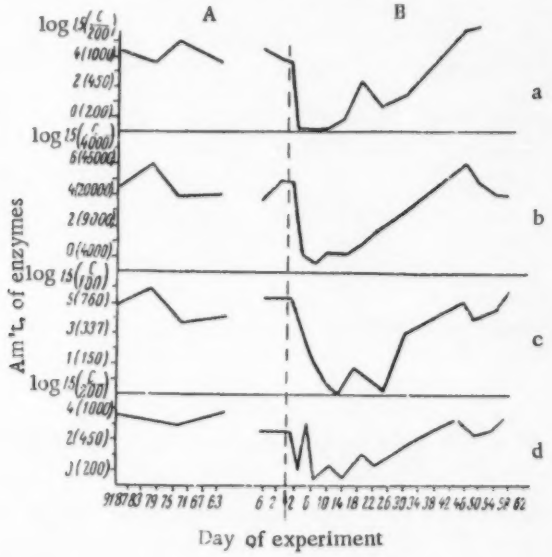


Fig. 2. Content of enzymes in the solid fraction of the secretion from a transplanted segment of intestine. A) Before severing the neurovascular bundle; B) after severing it; a) enterokinase; b) phosphatase; c) invertase; d) peptidase. The arrow indicates when the neurovascular bundle supplying the abdominal transplant was cut. The graduations on the ordinate axis refer to logarithmic values; the enzyme concentrations are given in parentheses (C), i.e., as the number of arbitrary units of activity in 1 g of the solid content of the secretion.

other enzymes (peptidase, invertase, lipase) began to fall 2-3 days after "denervation," and their total absence from the secretion was not observed for all the dogs. The enzyme content of the secretion began to rise again after 8-10 days, and had attained the initial levels after 1-2 months.

Transection of the neurovascular bundle of a transplanted segment had no perceptible effect on the amount and enzyme content of the secretion from the Thiry preparation. Nor was there any appreciable difference in the general condition of the animal.

Similar effects were found for intestinal segments "denervated" by the second method.

With either of the "denervation" techniques, paralytic secretion was associated with an abrupt fall in the concentration of the enzymes, as well as in the absolute amount secreted in unit time, notwithstanding the marked increase in the volume of secretion. The amount of enzymes secreted rose gradually with time, but remained considerably lower than from the Thiry preparation [11].

The differences between the secretion from isolated intestinal segments "denervated" in different ways persisted as time went on. The subcutaneous transplant secreted considerably less liquid and solid secretion a month after transection of the neurovascular bundle than before this operation. At still greater intervals of time (observations were continued on 4 dogs for over 3 years), the amount of secretion produced fluctuated slightly, but remained at a general low level, with even a certain tendency to fall still lower (Table 1).

TABLE 1

Amount of Secretion Produced from a Thiry Isolated Intestinal Segment and from a Transplanted Segment of the Upper Part of the Jejunum of Dog No. 1. Mean Values in g for 5 Hours

When experiments performed	Thiry intestinal segment		Transplanted segment	
	fluid part	solid part	fluid part	solid part
Before "denervation"	5.50	1.32	1.32	0.81
Time after "denervation"				
1 month	5.03	1.16	0.18	0.21
1 year	1.90	1.90	0.22	0.22
1½ years	2.68	1.02	0.17	0.36
2 years 5 months	1.28	1.08	0.11	0.24
2 years 8 months	2.59	0.91	0.09	0.19
2 years 11 months	1.31	0.89	—	—
3 years 3 months	1.64	1.36	0.11	0.16

The more long-term changes in secretion from segments "denervated" by transection of mesenteric nerves, followed by painting the vessels with phenol, proceeded rather differently, at 1-2 months after "denervation" (Table 2). Paralytic secretion had disappeared by the end of the month, but the amount of secretion continued to diminish, falling to a minimum at the end of the second month. It then began to rise slightly, both for the fluid and the solid fractions, but did not reach the same levels as for the Thiry segments, and continued at a low level.

The enzyme content of the secretion from intestinal segments "denervated" by both methods remained about the same, for 1-2 months, as before "denervation," or as from Thiry segments. The concentration of the enzymes remained at the same level over the remainder of the period of observation (Table 3).

The lower enterokinase content of the secretion from "denervated" segments is due to these having been formed from lower levels of the jejunum than were the Thiry segments.

Enzyme secretion over $1\frac{1}{2}$-2 months varied parallel with the variations in the amount of secretion produced, or, more closely, with the amount of the solid fraction. It was very low after "denervation," particularly from the transplanted segment, over the whole of the period of observation.

TABLE 2

Amount of Secretion Produced by a Thiry Segment and by a Segment of the Upper Jejunum Denervated by Transection of the Mesenteric Nerves, in Dog No. 2.

Mean values in g for 5 hours

Time after operation	Thiry segment		"Denervated" segment	
	fluid part	solid part	fluid part	solid part
1 month	2.38	0.86	0.29	0.80
2 months	1.52	1.12	0.02	0.13
1 year	1.33	0.84	0.24	0.39
1 year 4 months	1.32	0.71	0.15	0.40
1 year 11 months	1.54	0.73	0.18	0.46

The secretion of the solid part of the juice produced by Thiry segments (Tables 1, 2, and 3), and of the enzymes contained therein, settled down to a steady level over the whole of the period of observation, after recovery from the operational trauma (10-14 days). Secretion of fluid juice was fairly high for some time (up to 6 months for some dogs), then fell by $1\frac{1}{2}$ to 2 times this level, and varied very little thereafter, over a few years.

TABLE 3

Secretion of Enzymes by Isolated Thiry Transplanted Segments of the Upper Jejunum of Dog No. 3.

Mean values in arbitrary units

When experiments performed	Entero-kinase	Phos-phatase	In-vertase	Pepti-dase	Lipase
	Thiry segment				
Content of enzymes in one g of solid fraction of secretion					
Before "denervation" of the transplanted segment	4 280	38 000	700	637	173
3 weeks "after denervation"	4 500	29 900	590	572	170
After 7 months	2 940	30 450	451	457	114
After 2 years 7 months	—	41 000	796	386	161
Amount of enzymes secreted per hour					
Before "denervation" of the transplanted segment	855	7 770	161	131	34
3 weeks "after denervation"	620	5 640	111	103	32
After 7 months	1 060	7 700	108	117	28
After 2 years 7 months	—	6 800	143	69	30

TABLE 3 (continued)

When experiments performed	Entero-kinase	Phos-phatase	In-vertase	Pepti-dase	Lipase
Content of enzymes in one g of solid fraction of secretion					
Before "denervation"	1 100	24 960	723	604	162
3 weeks "after denervation"	1 000	22 900	375	435	73
After 7 months	2 000	27 600	674	752	292
After 2 years 7 months	1 680	—	375	590	166
Amount of enzymes secreted per hour					
Before "denervation"	301	6 080	181	185	33
After "denervation"	20	463	3	10	2
After 3 weeks*					
After 7 months	60	805	16	26	8
After 2 years 7 months	39	—	18	24	10

* Numbers omitted on original page.

Five dogs were killed 1-1½ years after formation of "denervated" segments, and the mucous membrane was examined histologically, and for the presence of enzymes.*

We could find no differences between the contents of intestinal enzymes of the mucous membranes of the Thiry preparations and of the "denervated" segments (by both methods), and of those of sections of intestine anatomically resembling the segments (Table 4).

TABLE 4

Enzyme Content of 1 g of Mucous Membrane from the Intestine and from Isolated Intestinal Segments (Arbitrary Units) Dog. No. 4

Part of intestine studied	Entero-kinase	Phos-phatase	Invertase
Above the level from which isolated segments formed	450	15 000	470
Thiry segment	500	23 000	620
Segment "denervated" by transection of the mesenteric nerves and painting the vessels with phenol	500	30 000	500
Below the level from which isolated segments formed	300	30 000	570

The transplanted segment was macroscopically markedly atrophied, its length and diameter having been reduced to about half the initial sizes. Microscopic examination showed well-marked degenerative changes in the mucous membranes and in the intramural nervous plexi.

In contrast to this, the segments "denervated" by transection of the mesenteric nerves, and left in the abdominal cavity, were macroscopically unchanged, and the histological signs of degeneration were much less pronounced. We found nerve fibers in the mesentery, distal to the transection, which were histologically of normal structure, and which were apparently functional. This indicates that nervous connections with the central nervous system were reestablished in the "denervated" segment of intestine.

* The morphological studies were conducted by N. A. Nilova, S. I. Matveeva, and E. A. Rudik.

Our results show that the enzyme-secreting function of the intestine is profoundly affected by severing connections with the central nervous system. Production of enzymes is greatly depressed during the phase of paralytic secretion. It seems probable that termination of paralytic secretion is connected with ingrowth of new nerves into the intestinal segment. This is in complete accord with the known rate of growth of regenerating nerves, of about $1^1/_2$ mm per diem [3], and with the time taken for the enzyme content of the secretion to revert to its initial level ($1-1^1/_2$ months).

The degree of restoration of the secretory function of the intestine, and the extent of the degenerative processes observed in "denervated" segments are connected with the nature of the newly ingrowing nerve fibers.

At considerable times after "denervation," when the ingrowing nerves come from the subcutaneous tissues, the mucous membranes of the intestinal segment regain the ability of secreting enzymes in concentrations usual for the intestine, but in only very small amount. A somewhat fuller degree of recovery of the secretory function of "denervated" segments is seen when the ingrowing nerve fibers come from the abdominal organs. The secretion produced by such segments has approximately the same concentration of enzymes as that from segments newly innervated from subcutaneous tissue, although the amount secreted is somewhat greater, but is still much less than from segments with intact nervous connections.

LITERATURE CITED

[1] L. G. Voronin, Izvest. Inst. im. Lesgafta, 21, No. 1-2, 3 (1938).

[2] B. Glasson and P. Anokhin. Byull. VIEM, 1935, No. 1, 15.

[3] Yu. M. Zhabotinsky, Normal and Pathological Morphology of Vegetative Ganglions,* Acad. Med. Sci. USSR Press, Moscow, 1953.

[4] A. I. Levina, Cross-Suture of Nerves, Synpathetic and Cranial Systems.* Experimental Studies, Kazan.

[5] L. G. Merkulov, Byull. VIEM, 1936, No. 2, 16.

[6] L. G. Merkulov, Fiziol. Zhur. SSSR, 20, No. 1, 132 (1936).

[7] S. Ya. Mikhlin and I. B. Kuvaeva, Biokhimiya, 19, No. 4, 437 (1954).

[8] L. A. Orbeli, Fiziol. Zhur. SSSR , 5, No. 1-3, 322 (1922).

[9] M. I. Razumov, Voprosy Pitaniya, 11, No. 4, 18 (1952).

[10] L. S. Fomina, Trudy AMN SSSR, 1951, Vol. 13, Voprosy Pitaniya, No. 1, 1307.

[11] L. S. Fomina, Voprosy Pitaniya, 12, No. 3, 22 (1953).

[12] L. S. Fomina, S. Ya. Mikhlin, and G. K. Shlygin, Biokhimiya, 1952, No. 2, 134.

[13] G. K. Shlygin, Uspekhi Sovremennoi Biol., 33, No. 1, 14 (1952).

[14] G. K. Shlygin, Biokhimiya, 15, No. 6, 509 (1950).

[15] W. Borchardt, Pflugers Archiv. f. d. ges. Physiol., 1927, B. 215, H. 3, S. 402.

[16] B. Molnar, Deutsche med. Schr. , 1909, N. 38, S. 1384.

[17] H. N. Pierce, E. S. Nasset and J. R. Murlin, J. biol. chem., 1953, Vol. 108, p. 239.

[18] R. D. Wright, M. A. Jennings, H. W. Florey and R. Liuru, Quart. J. Exptl. Physiol., 1940, Vol. 30, N. 1, p. 73.

* In Russian.

EFFECT OF ASCARID TOXIN ON EXCITABILITY OF INTESTINAL CHEMOCEPTORS

I. A. Edelshtein

From the Pathophysiological Laboratory (Director: Assistant Prof. I. A. Edelshtein), Vitebsk Veterinary Institute (Director: Prof. V. F. Lemesh)

(Received October 24, 1955. Presented by V. N. Chernigovsky, Member Acad. Med. Sci. USSR)

We have shown in an earlier paper [9] that contact of chemoceptors with helminthic toxins causes reflex changes in blood pressure, respiration, and other processes.

The object of the present research was to ascertain whether the physiological properties of interoceptors are affected by contact with helminthic toxins.

EXPERIMENTAL METHODS

We performed 22 experiments on cats under urethane narcosis. We studied reflex changes in blood pressure and respiration following stimulation of intestinal receptors with acetylcholine and carbon dioxide, before and after injecting ascarid toxin into the intestinal blood vessels. For this purpose, an intestinal loop isolated from the systemic circulation was prepared. An aqueous extract of Ascaris suum was prepared by E. N. Pavlovsky's procedure, and 1 ml of extract, diluted from ten- to a thousand-fold, was injected into the appropriate vessel. In using aqueous extracts of the worms we realized that these were not fully equivalent to the isolated helminthotoxins. It is, however, generally accepted that the extracts have a similar composition to the products of digestion of the worms in the intestine of the host. Stimulation with carbon dioxide was achieved by changing the perfusion fluid to Ringer-Locke solution saturated with CO_2. Acetylcholine solution was injected into the intestinal vessels.

EXPERIMENTAL RESULTS

The first series of experiments dealt with alterations in the reaction to carbon dioxide. The following findings emerged. Before treatment with ascarid extract, carbon dioxide produced its usual effect, viz., reflex rise in arterial pressure and in respiration. After the extract had been applied, the same concentration of carbon dioxide produced the opposite effect, viz., fall in blood pressure and depression of respiration (see Figure); in some of the experiments this change did not appear immediately after introduction of the extract. At first, we observed only weakening or disappearance of the reflexes, or a diphasic pressor-depressor reaction, and this was followed by fall in blood pressure and depression of respiration. The normal reaction to CO_2 could not be restored by prolonged perfusion with Ringer-Locke solution, for 2-3 hours.

In the second series of experiments we studied the effects on the reflex reactions to acetylcholine (at dilutions of 1×10^{-3} to 1×10^{-5}) after introduction of ascarid extracts.

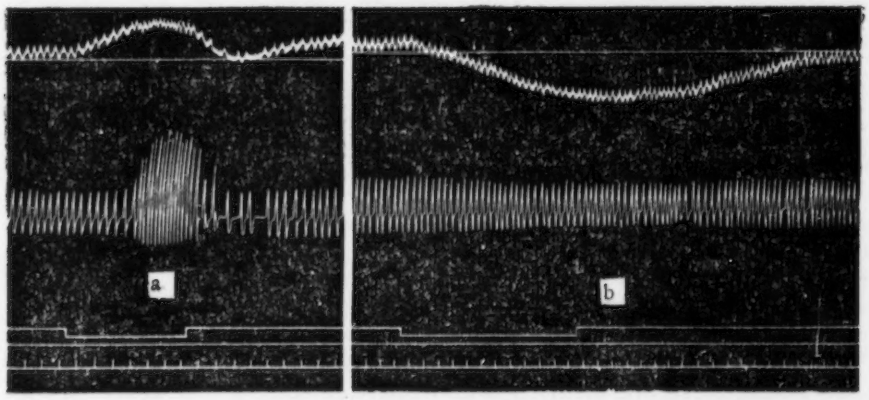
These experiments showed that stimulation of the chemoceptors with acetylcholine after exposure to ascarid toxin gave heightened blood pressure and respiration reflexes. In addition to this effect, we observed in some of the experiments a change in the nature of the reflexes: instead of a monophasic pressor reaction, we found a diphasic pressor-depressor reflex.

Thus all our experiments showed that the effect of toxic ascarid constituents on the chemoceptors of the intestinal blood vessels was to change the degree and the nature of reflex reactions of the vasomotor and respiratory centers to such stimuli as carbon dioxide and acetylcholine.

Alterations of reflexes from intestinal chemoceptors have been reported by a number of authors for various experimental conditions (V. N. Chernigovsky [8], V. A. Lebedeva and V. N. Chernigovsky [6], M. A. Erzin [1], V. A. Lebedeva [2, 3, 4, 5], F. A. Levtova [7], and others).

According to these authors, the alterations in the excitability of the chemoceptors are connected with primary reactions of nerve endings to disturbances of metabolism of the organ.

V. A. Lebedeva concluded, from her numerous researches, that the depressor response to carbon dioxide found under conditions of impaired metabolism should be regarded as a consequence of enhanced excitability of the receptors. In our experiments poisoning with ascaris extracts caused a depressor reflex response to hypercapnic Ringer-Locke solution, and an intensified response to stimulation with acetylcholine. It follows that in both series of experiments the excitability of the chemoceptors of the intestinal blood vessels was raised, as a result of the toxic action of the ascaris extractives. It may be supposed that the helminthic toxins provoke a profound disturbance of the metabolism of the intestinal cells.



Changes in reflex blood pressure and respiration responses to stimulation of intestinal chemoceptors with carbon dioxide (A) before, and (B) after, action of ascaris extract. Explanation of tracings (from above down): blood pressure (mercury manometer), initial blood pressure level, respiration, stimulation signal, zero level of the manometer, time marker (5 second intervals).

We shall in our future researches examine the effects of helminthic toxins on the metabolism of the tissues of the receptor field.

LITERATURE CITED

[1] M. A. Erzin, Byull. Eksptl. Biol. i Med., 23, No. 6, 426-429 (1947).

[2] V. A. Lebedeva, Byull. Eksptl. Biol. i Med., 34, No. 11, 17-21 (1952).

[3] Ibid, Byull. Eksptl. Biol. i Med., 35, No. 4, 5-10 (1953).

[4] Ibid, Byull. Eksptl. Biol. i Med., 38, No. 11, 19-27 (1954).

[5] Ibid, Byull. Eksptl. Biol. i Med., 39, No. 1, 22-27 (1955).

[6] V. A. Lebedeva and V. N. Chernigovsky, Byull. Eksptl. Biol. i Med., 31, No. 3, 153-158 (1951).

[7] F. A. Levtova, Role of the Nervous System in the Pathogenesis and Treatment of Tuberculosis,* p. 24, Moscow, 1954.

[8] V. N. Chernigovsky, Afferent Systems of Internal Organs.* Kirov, 1953.

[9] I. A. Edelshtein, Uchenye Zapiski Vitebskogo Vet. Inst., 13, 142-146 (1954).

* In Russian.

EFFECT OF THE THYROID HORMONE ON THE EVACUATORY FUNCTION OF THE STOMACH*

S. G. Genes and N. G. Lesnoi

From the Department of Pathological Physiology (Director: Prof. S. G. Genes, Honored Scientific Worker), Ukrainian Institute of Experimental Endocrinology (Director: Candidate of Medical Sciences S. V. Maksimov)

(Received December 23, 1955. Presented by V. N Chernigovsky, Member Acad. Med. Sci. USSR)

Insufficient work has been done on the effect of the thyroid hormone on the evacuatory function of the stomach [3, 4, 5, 6]. The study of this problem has been complicated by the great variability in the evacuatory function of the stomach, by the deficiencies of the generally applied method for its quantitative evaluation, by its relative insensibility, and by the insufficient time available for observations (the bulk of water introduced is evacuated into the intestine within 30-45 minutes).

We have frequently noted the variability of the evacuatory function of the stomach. On one occasion we found that no more than 5-10 ml of 300 ml of water introduced into a dog's stomach passes into the duodenum during the first 15 minutes, while during the following 15 minutes 100-200 ml are evacuated, and on another occasion we found the opposite effect. Such fluctuations are, however, observed only when the observations are made for short periods of time. They are less pronounced when the period of observation is 1 hour, and still less so when it is 3-4 hours [2]. For this reason, evaluation of the state of the evacuatory function of the stomach should not be based on observations of short duration.

We have examined the effect on the evacuatory function of the stomach of excess or deficiency of thyroid hormone (in the same animals), using the method of S. G. Genes [2].

EXPERIMENTAL METHODS

The experiments were performed on 4 dogs with Basov gastric fistulae: Julia, Tyulpan, Mishuk, and Bars. Volumes of 500, 700, and 800 ml of water were introduced into the stomach at 15 minute intervals. A quarter or a third of the water passed into the duodenum over a 15 minute period; only rarely was the amount somewhat greater or smaller than this.

The dogs were taken out to urinate at hourly intervals, in order that filling of the bladder should not affect the evacuatory function of the stomach. The weight of the animals was still slightly above the initial value at the end of the 4-hour experimental periods, but was back to the initial figure after 2-3 hours, and only rarely after 4 hours.

EXPERIMENTAL RESULTS

Our control experiments showed that the volume of water passing from the stomach into the duodenum was about the same during each hour (Tables 1-4). The volume evacuated differed for different dogs, this being to some extent due to differences in the amounts introduced into the stomach. The water loading did not affect the composition of the blood; hourly analyses showed that the hemoglobin content remained constant.

The condition of the evacuatory function of the stomach was studied at the beginning and the end of a period of feeding with raw thyroid gland.

The amount of water leaving the stomach rose somewhat (by 7, 11, 13, and 19%) at the beginning of the period of thyroid feeding.

*Read on December 13, 1954 at the Joint Meeting of the Kharkov Endocrinological Society and the Kharkov Branch of the Ukrainian Association of Physiologists, Biochemists, and Pharmacologists.

TABLE 1

Passage of Water from the Stomach to the Duodenum of the Dog Tyulpan (weight 25 kg)

Experimental series	No. of experiments	ml of water transferred during					Total water transferred during 4 hrs, as %
		1st hour	2nd hour	3rd hour	4th hour	4 hours	
Control, January 27 to March 6.	7	1089	1201	1140	1160	4590	100
Daily feeding with 50 g portions of raw thyroid gland (March 10-31)	4	1289	1261	1276	1270	5086	111

TABLE 2

Passage of Water from the Stomach to the Duodenum of the Dog Mishuk (weight 24 kg)

Experimental series	No. of experiments	ml of water transferred during					Total water transferred during 4 hrs, as %
		1st hour	2nd hour	3rd hour	4th hour	4 hours	
Daily feeding with 50 g portions of raw thyroid gland (March 9-April 7):							
March 10-20	4	1245	1325	1319	1178	5067	119
April 3-7	2	1170	1380	1195	1253	4998	117
After discontinuing feeding with raw thyroid gland April 10-24	3	893	1177	1227	1358	4655	109
Daily administration of 3-6 g of methylthiouracil (April 27-July 24):							
May 6-15	3	997	1198	1213	1208	4616	108
May 19-June 9	7	615	992	1021	1024	3582	84
June 18-July 24	8	627	761	872	907	3167	74
After discontinuing 6-methylthiouracil administration (July 24-Sept 30)							
July 28-September 30	9	727	897	1030	1025	3679	86

By the end of this period, the evacuatory function of the dog Bars had risen by a further 11%, remained unchanged for Mishuk, and fallen somewhat for Julia, becoming only 7% above normal (there were no data for Tyulpan over this period).

Over a period of about 3 weeks after discontinuing thyroid feeding, the evacuatory function of the stomach of the dogs Julia and Bars had returned to the normal values, while that of Mishuk was still somewhat elevated.

Prolonged oral administration of 6-methylthiouracil caused a 3-5 fold increase in weight of the thyroid glands, and led to the development of various trophic changes (eczema, falling out of the hair, skin ulcerations). The evacuatory function of the stomach was depressed, but only to a small degree, and differently for each dog: in Bars by 3 and 9%, in Julia by 6 and 19%, in Mishuk by 16 and 26% (after an initial rise of 8%).

Augmentation of the evacuatory function took place only very slowly after discontinuation of the 6-methylthiouracil.

TABLE 3

Passage of Water from the Stomach to the Duodenum of the Dog Julia (weight 18 kg)

Experimental series	No. of experiments	ml of water transferred during					Total water transferred during 4 hrs, as %
		1st hour	2nd hour	3rd hour	4th hour	4 hours	
Control Jan 30-March 7	7	594	794	712	600	2700	100
Daily feeding with 50 g portions of raw thyroid gland (March 9-April 7)							
March 11-21	4	774	854	716	718	3062	113
April 4-8	2	880	800	698	610	2888	107
After discontinuing feeding with raw thyroid gland							
April 11-28	4	510	749	738	631	2628	97
Daily administration of 2.25-3.25 g of 6-methylthiouracil (April 28-July 9):							
May 4-June 16	13	466	648	619	731	2536	94
June 27-July 4	2	455	400	685	645	2185	81
After thyroidectomy July 9							
July 14-August 1	6	519	639	614	661	2433	90
August 8-October 27	7	400	519	545	589	2054	76
Daily feeding with 10-20 g portions of raw thyroid gland (October 27 to November 17)							
December 3-17	4	650	736	696	491	2573	95

We performed a thyroidectomy on Julia and Bars, in order to elucidate the effect of the goiter and the hypothyroidism produced by 6-methylthiouracil administration. This caused a slight lowering of evacuatory function at first (by 1 and 10%), with a somewhat greater one (by 24 and 33%) after 20-30 days. It may be concluded that prolonged administration of high doses of 6-methylthiouracil depresses thyroid function, and the weakening of the evacuatory function of the stomach observed in this condition is ascribable to the hypothyroidism produced. Subsequent feeding of the thyroidectomized dogs with raw thyroid glands (10-20 g daily) gave a rapid normalization of evacuatory function.

Excess or deficiency of thyroid hormone has an effect on all the functions of the organism, mediated basically through various parts of the central nervous system (for references see S. G. Genes[1]).

In order to elucidate to what extent the effect of thyroid hormone on the evacuatory function of the stomach is mediated through the central nervous system, we put the dogs to sleep with the aid of Medinal. The results of these experiments are presented in Table 5.

The experiments showed that, in profound narcosis produced by Medinal, the evacuatory function of normal dogs is weakened, by 24, 39, and 42%, on the average, in 3 dogs, and raised by 6% in the fourth; the corresponding figures for three thyroid-fed dogs were 7, 11, and 42%, for 6-methylthiouracil-treated dogs 20, 25, and 36%, and for two thyroidectomized dogs 30 and 38%, below waking values.

These data permit the conclusion that excess thyroid hormone affects central nervous system regulation of the evacuatory function of the stomach to a much smaller degree than it does metabolic processes, with the connected functions of the cardiovascular and respiratory systems. Absence of thyroid hormone leads to considerable weakening of central nervous regulation of the evacuatory function of the stomach.

TABLE 4

Passage of Water from the Stomach to the Duodenum of the Dog Bars (weight 27 kg)

Experimental series	No. of experiments	ml of water transferred during					Total water transferred during 4 hrs, as %
		1st hour	2nd hour	3rd hour	4th hour	4 hours	
Control Jan 30-March 7	7	1299	1229	1315	1062	4955	100
Daily feeding with 50 g portions of raw thyroid gland (March 9-April 7)							
March 11-21	4	1525	1548	1214	1024	5311	107
April 4 and 8	2	1713	1643	1333	1133	5822	118
After discontinuing feeding with raw thyroid gland							
April 9-28	4	1171	1278	1364	999	4811	98
Daily administration of 3.25-6.5 g of 6-methylthiouracil (April 28-July 14):							
May 4-June 24	15	1166	1194	1280	1146	4786	97
July 4 and 14	2	985	1140	1070	1315	4510	91
After thyroidectomy (July 20):							
July 29-August 4	3	1097	1200	1325	1247	4869	99
August 10	1	450	840	915	1095	3330	68
August 18-October 27	6	632	798	956	898	3284	67
Daily feeding with 10-20 g portions of raw thyroid gland (October 27 to November 17)							
November 3-17	4	1383	1341	1350	843	4916	100

TABLE 5

Effect of Medinal-induced Sleep on the Evacuatory Function of the Stomach

Experimental series	State of animals	No. of experiments	Tyuplan		Mishuk		Julia		Bars	
			transfer of H_2O from the stomach after 4 hours							
			ml	%	ml	%	ml	%	ml	%
Control	Waking	5	4525	100	4319	100	2729	100	4967	100
	Sleeping	2	4805	106	2498	58	2088	76	3020	61
Thyroid feeding	Waking	4	5086	100	5066	100	3061	100	5311	100
	Sleeping	2	4727	93	2933	58	1768	58	4738	89
Administration of 6-methylthiouracil	Waking	10			3892	100				
		13					1811*	100	4786	100
		15								
	Sleeping	2			2490	64	1353*	75	4830	80
After thyroidectomy	Waking	6					2433	100		
		5							4687	100
	Sleeping	2					1513	62	3301	70

* Data from 3-hour experiments

Medinal narcosis leads to a much more rapid and pronounced development of depression of the evacuatory function of the stomach than does thyroidectomy. Much less water is passed from the stomach to the duodenum of narcotized dogs which had been subjected to prolonged thyroid feeding than in the waking state. Medinal administration does not, however, fully abolish the action of thyroid hormone. Thus, evacuation in the thyroid-fed dogs Mishuk and Bars during narcotic sleep was considerably greater (by 17 and 50%) than in the control series. It follows that, for the majority of the dogs, the central nervous system, having been modified by prolonged administration of thyroid hormone, gives a weaker response to Medinal than in normal animals. Higher doses of Medinal are required to induce sleep in hyperthyroid dogs than in the control experiments (0.24-0.27 g instead of 0.22 g per kg body weight). In spite of these elevated dosage levels, the stage of excitation was considerably prolonged in these animals, and their sleep was interrupted from time to time. They usually awoke after 3-4 hours. Induction of sleep was particularly difficult in the case of the dog Tyulpan, and his sleep was uneasy; a restless, drowsy condition supervened, lasting for days, and he finally died in this condition, in spite of administration of analeptics (camphor, caffeine, strychnine). Hypothyroid dogs reacted differently towards Medinal. Such dogs fell asleep after doses of Medinal not exceeding 0.2 g per kg body weight. The amount of water evacuated from the stomach during such sleep was much smaller than during the thyroid feeding period.

The inconsiderable strengthening of the evacuatory function of the stomach observed during prolonged thyroid feeding is evidence of the great stability of this function, particularly if we consider the marked rise in pulse rate (nearly 3-fold) and respiratory rate (nearly 7-fold), and the fall in weight (by 9-11%). This stability may be a result of the low sensitivity to thyroid hormone of the parts of the central nervous system concerned in the regulation of the evacuatory function of the stomach, and of the peculiarities of the vegetative ganglia of the gastrointestinal tract. The pronounced depression of the evacuatory function of the stomach found after administration of Medinal is evidence of the role of central nervous system regulation. Yet the fact that the evacuatory function retains 58, 61% or even more of its waking value during profound sleep necessitates an evaluation of the role of the vegetative system ganglia in the evacuatory function of the stomach. I. P. Pavlov wrote the following, in 1898: "The stomach plays an important part in the movement of food. It knows when it is filled, and, unless there is a pyloric obstacle, it expresses its contents."

Depression of evacuatory function in hypothyroidism and after thyroidectomy is evidence that the continuous action of thyroid hormone on the relevant parts of the central nervous system is essential for the normal functioning of the evacuatory mechanism of the stomach.

LITERATURE CITED

[1] S. G. Genes, Uspekhi Sovremennoi Biol., 35, No. 2, 229-256 (1953).

[2] S. G. Genes, Byull. Eksptl. Biol. i Med., 37, No. 4, 74-76 (1954).

[3] R. I. Levina, Eksptl. Med., 1938, No. 3, 35-42.

[4] L. G. Merkulov, E. V. Neshel , and E. N. Speranskaya-Stepanova, Fiziol. Zhur. SSSR, 30, No. 6, 714-722 (1941)

[5] P. Trendelenburg, Hormones, Their Physiology and Pharmacology,* Vol. 2, Moscow-Leningrad, 1936.

[6] G. Deusch, in : Handbuch der inneren Sekretion, Hrsg. von M. Hirsch, Bd. 1, S. L–1, Leipzig, 1932.

* In Russian.

AN INTESTINAL FISTULA TUBE

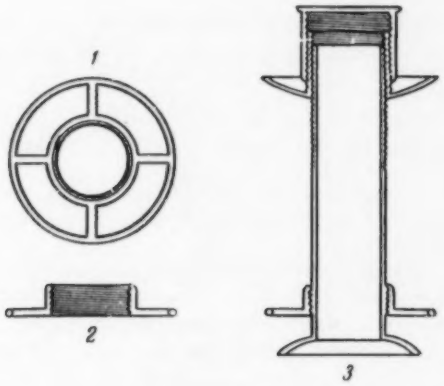
A. N. Bakuradze

From the Chair of Normal Physiology (Director: A. N. Bakuradze, Corresponding Member Acad Sci. Georgian SSR), Tbilisi Medical Institute

(Received February 4, 1956. Presented by V. N. Chernigovsky, Member Acad. Med. Sci. USSR)

Fistula tubes implanted into the jejunum frequently fall out, chiefly because the small diameter of the intestine necessitates the use of tubes with an internal flange of small diameter.

To prevent dislodgement of the tube after suturing it in place, we screw down an additional disc, close to the internal flange. This is in the form of a cross contained in a circle, of diameter slightly greater than that of the internal flange (see Figure). In this way, the intestinal wall is between the inner flange and the additional device.



Device preventing dislodgement of a fistula tube from the thin intestine.
1) Additional disc (seen from above); 2) section of same; 3) vertical section of fistual tube.

When the outer part of the tube is taken out to the exterior, the additional disc is in direct contact with the inner surface of the abdominal wall, which assures firm fixation of the inner flange of the tube within the lumen of the intestine. In view of the open nature of the additional disc, adhesions between the outer surface of the intestine and the parietal peritoneum form without hindrance.

So constructed, the fistula tube (see Figure) will never become dislodged.

SOME POSSIBLE FORMS OF INTENSIFICATION OF SLOW WAVES IN THE ELECTROENCEPHALOGRAM

N. P. Bekhtereva

From the Prof. Polenov Leningrad Scientific Research Neurosurgical Institute (Director: Prof. V. N. Shamov, Member Acad. Med. Sci. USSR)

(Received June 17, 1955. Presented by P. S. Kupalov, Member Acad. Med. Sci. USSR)

In addition to the ordinary electroencephalograms used in clinical practice, frequent use is now made of electroencephalograms recorded from patients under determined conditions of cerebral activity.

In our research, which was undertaken at the suggestion of Prof. A. V. Lebedinsky, we recorded electroencephalograms while subjecting the patient to the action of indifferent light and sound signals, and then to conditioned light and auditory stimuli, and to their various combinations. The recording instruments were an oscillographic electroencephalograph and a Kaiser ink-writing electroencephalograph.

The conditioned reflexes were elaborated by the movement-speech procedure. We examined 90 patients suffering from tumors, tumor-like conditions, and acute closed traumatic injuries of the brain. In all we made 245 recordings.

It has been shown by a number of workers[2, 3, 5, 6, 9, 11] that various alterations in the electroencephalogram rhythms may be observed when the patients are exposed to stimulation.

In the present paper we report one of the possible variants of intensification of slow waves, which was very briefly described by V. S. Rusinov in 1948 [8] as a hypersynchronization effect.

In 50 examinations of 32 patients, the slow waves arising in response to exhibition of positive signals took the form of waves of large amplitude (up to 200-250 μv), diminishing later, of a periodicity of 0.2 to 0.6 seconds. In some of the patients the waves were at first of an angular form, and later assumed the usual rounded contours. These waves were distinguished by a certain rhythmicity and regularity of potentials, as compared with the ordinary slow waves.

When we first observed this phenomenon we were not convinced of the reality of its physiological origin. Considering the form and the nature of the waves, a purely physical explanation of the phenomenon seemed to be too tempting. As further material accumulated, however, we were obliged to abandon this explanation, the more so as the damping down of the oscillations was not always observed. We only became convinced of the physiological nature of the waves after we found that their appearance, the duration of their latent period, etc., depended on the condition of the subjects under examination.

Each burst of slow activity was 3-8 waves, and lasted for from 1 to 3-5 seconds, most often beginning and ending during the time of action of the signal, which was discontinued as soon as the patient gave a motor response.

The effect was very pronounced in certain of the patients, and in these cases its duration was also increased, the slow rhythmic waves persisting for 1-2 seconds after cessation of the action of the stimulus. In some cases the reaction consisted of two or even three bursts of slow wave activity, appearing in quick succession at intervals of 1-1.5 seconds, and usually only the first of these bursts appeared during the action of the signal (Figure 1). In two cases only did the rhythmic slow waves appear 4-5 seconds after the signal and its motor response reaction. Such effects were observed in patients during the first ten days after operation. Similar slow waves appeared in three patients after squeezing the bulb without previous elaboration of the conditioned reaction, but they were less pronounced.

High amplitude rhythmic slow waves were encountered in patients whose electroencephalograms showed pathological slow waves in the background rhythm, as well as in patients whose resting encephalogram showed no slow waves. When inhibitory signals were applied, the rhythmic slow waves were usually encountered when differentiations were being disrupted. Only in one case did the slow waves appear on exhibition of an inhibitory

signal in the absence of any signs of disruption of differentiation. It hence seems that the form of slow activity observed in association with positive signals is a manifestation of the stimulatory process itself, arising under some special conditions, or else of inhibition of a different type of internal inhibition.

The hypersynchronization effect was observed by us chiefly in the frontal regions of the brain, as was also found by V. S. Rusinov [7]; in some cases, however, it was found for the whole of the cerebral cortex. When we applied visual conditioned signals, the rhythmic slow waves were most pronounced in the posterior parts of the frontal lobe and with the occipital lead. In some patients the slow waves were only recorded with occipital or parietal leads. Irrespective, however, of whether the motor response to the conditioned signal was made by the right or the left hand of the patient, the slow waves were more frequently registered from the right cerebral hemisphere.

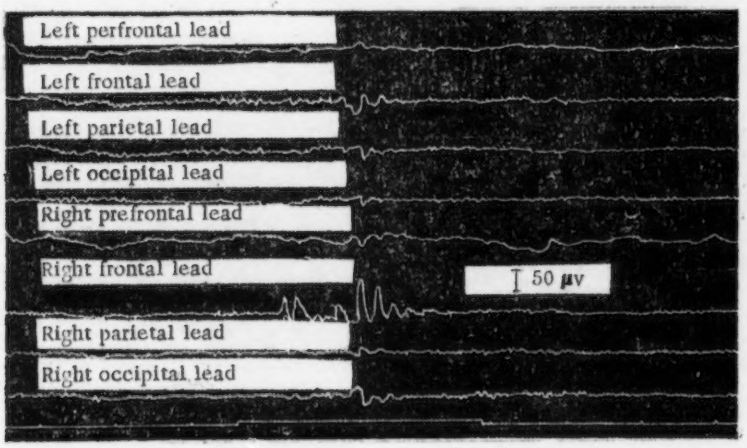


Fig. 1. Electroencephalogram of the patient M., suffering from an angioma of the right cerebellopontile angle.
Explanation of tracings (from above down): electroencephalogram of the left prefrontal region, of the left posterior frontal region, of the left parietal region, of the left occipital region, of the right prefrontal region, of the right frontal region, of the right parietal region, of the right occipital region, conditioned signal marker, for positive visual and auditory complex stimuli applied simultaneously.

Electroencephalograms of this type may be a consequence of the spreading of a single process over a large group of cells, viz., of the synchronized action of a large group of neurones. Such a process could equally well be a diffused stimulatory one, or an inhibitory process. Considering, however, that a characteristic feature of these waves is, apart from their high amplitude and their relative rhythmicity, the increase in their periodicity, which indicates a lowering of the lability of the nervous structures participating in the reaction, which should, in all probability, favor inhibition [1, 2, 12]. The localization of the slow waves chiefly in the right hemisphere, even when the motor reaction is effected by the left hand of the patient, puts the mechanism of interhemispheric induction among the possible explanations of the phenomenon. The reason for the regularity of the rhythmicity of the waves is not clear. We can only refer it to the facility with which rhythmic slow activity can be elicited under conditions which involve alterations in the interrelations of nervous processes in the cerebral cortex of animals [5, 13].

We observed a phenomenon, in the same research, which owing to its superficial similarity to the above-described phenomena was at first thought by us to be related to them.

After exhibition of conditioned signals, groups of waves appeared on the electroencephalograms of some patients, which were of the same form as the high voltage slow activity described above, but with a shorter period of oscillation, the frequency being of the order of the alpha rhythm. This was seen in its most typical

form in patient K. In the early postoperational period, his electroencephalogram was very smooth, with no high-potential waves, and the delta and alpha waves seen before the operation were no longer in evidence. The case history of this patient showed that his postoperational condition was grave. Up until the time when the first electroencephalogram was recorded the patient was in a profoundly inhibited state, being very slow to answer questions, and giving monosyllabic answers, and he was very feeble and apathetic. Considering the clinical data together with the electroencephalograms, we came to the conclusion that the latter reflected an irradiation of inhibition throughout the whole of the cerebral cortex.

We were able to observe the appearance of alpha waves in response to positive conditioned stimulation, and after a protracted latent period. Further attempts at stimulation, involving the simultaneous application of two or more positive stimuli, elicited a retardation of the oscillations, with the appearance of large delta waves (Figure 2). Towards the end of the examination of this patient a single positive stimulus was sufficient to elicit waves of a greater period than alpha waves. Under these conditions stimultaneous exhibition of a positive stimulus and an inhibitory signal led to a rise in frequency of the waves (Figure 3). We should add, however, that a breakdown of differentiation took place after this, and we were not able to reestablish it subsequently.

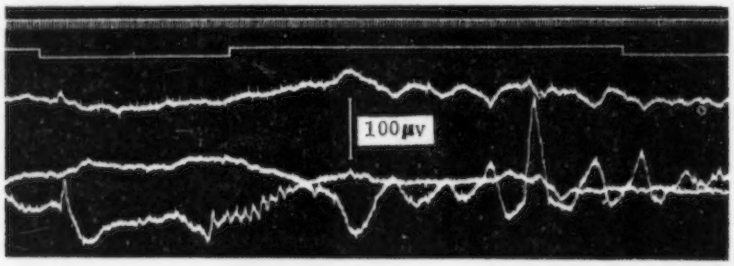


Fig. 2. Electroencephalogram of the patient K., recorded April 8, 1955. A recurrence of a tumor of the right temporal-parietal lobe was diagnosed. The cyst was evacuated on March 19, 1955, without removing the tumor. Explanation of tracings (from above down): time marker (1 second), signal indicating time of exposure to positive light (blue, red) conditioned signals, electroencephalograms.

How can we explain the appearance of an alpha rhythm in response to a positive conditioned signal, in the total absence of such waves in the background rhythm?

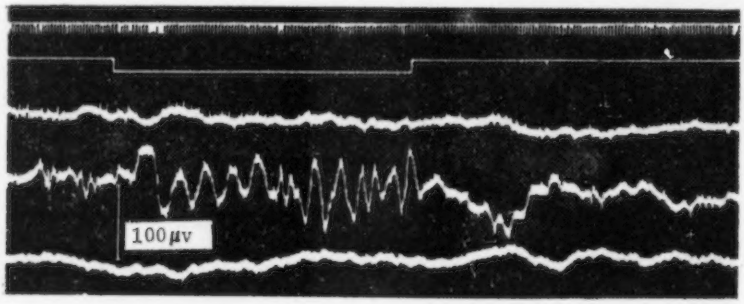


Fig. 3. Electroencephalogram of the patient K., recorded April 12, 1955. Explanation of tracings (from above down): time marker (1 second), signal indicating time of exposure to conditioned signals, viz., a visual complex positive stimulus and an auditory inhibitory stimulus, applied simultaneously, electroencephalograms.

As is known, the alpha rhythm appears in normal subjects both when the activity of the cortical cells is intensified and when it is depressed, with change in the conditions of functioning of the brain. The alpha rhythm thus represents a form of bioelectric activity which is manifested in certain definite functional states of the brain [2], and which characterizes the excitability of the cortical nerve cells [3, 4].

Exhibition of a conditioned signal exalted the excitability of the nerve cells to a level sufficient for the appearance of alpha waves. As has been shown, further intensification of the stimulus led to increase in the period of oscillation.

Why did the presentation of positive signals in most cases elicit slow oscillations of the delta wave type, although it was possible under similar conditions to observe the appearance, or the strengthening, of alpha waves?

I. P. Pavlov [8], in examining the interaction of nervous processes in the central nervous system by the conditioned reflex method, demonstrated the limiting role of an inhibitory process in the spread of an excitatory one. He emphasized that a delimiting function of this sort can only be fulfilled by a sufficiently powerful inhibition. Stimulation concentrated in certain definite regions of the cerebral cortex, although these vary from moment to moment, does not, in spite of constant broad irradiation, stimulate the vast majority of the nerve cells to a state of activity. This lowers the possibilities of development of superlimiting inhibition.

A. I. Roitbak [10] has, on the basis of the laws discovered by N. E. Vvedensky, also related dispersion of potential arising during direct stimulation of the cortex to its concentration by an inhibitory process.

V. S. Rusinov [11] has stressed the basic similarity of the mechanisms concerned in initiation of the fundamental forms of bioelectric activity of the brain. In considering the origin of alpha, delta, and beta waves, he believes that their periodicity depends on the number of cellular elements coming into play.

It is possible that the frequency of the oscillations observed by us was determined by the relative strengths of the stimulatory and inhibitory processes, and by the extent of irradiation of the stimulatory process in our patients. The truth of such an assumption is supported by M. N. Livanov's data [4] showing normalization of the electroencephalogram rhythms when the experimental animals were given bromide, as well as by our observation that the period of the slow waves is shortened when an inhibitory signal is added to a positive stimulus. The presence of a state of profound inhibition in the central nervous system of the patient K, at first limited excitations arising therein, and led to the appearance of alpha waves. In the given case, the role of the delimiter of excitation, was, in distinction from the normal conditions, fulfilled by superlimiting inhibition, possibly superadded to inductive inhibition. In other cases, a weak inhibitory process permitted the irradiation of excitations to a large number of nerve elements, evidently so causing an intensification of superlimiting inhibition of these elements, which found its physiological expression in the lengthening of the period of the oscillations.

LITERATURE CITED

[1] N. E. Vvedensky, Selected Works,* No. II, Leningrad, 1951.

[2] N. V. Golikov, Uchenye Zapiski LGU, Ser. Biol. Nauk, 1950, No. 123 (22), 202-228.

[3] M. N. Livanov, Zhur. Obshchei Biol., 5, 1, 8-42 (1944).

[4] M. N. Livanov, Pavlov's Teachings in Theoretical and Practical Medicine, pp. 124-125, Moscow, 1951.

[5] M. N. Livanov and K. L. Polyakov, Izvest. Akad. Nauk SSSR, Otd. Biol. Nauk, 1945, No. 3, 286-307.

[6] V. E. Maiorchik and B. G. Spirin. Voprosy Neirokhirurg., 15, No. 3, 3-11 (1951).

[7] V. E. Maiorchik and V. S. Rusinov, Trudy Inst. Neirokhirurgii im. Burdenko, pp. 64-77, Moscow, 1948.

[8] I. P. Pavlov, Lectures on the Activity of the Cerebral Hemispheres,* Vol. 4, Moscow, 1951.

[9] I. A. Peimer, Zhur. Nevropatol. i Psikhiatr., 55, 161-170 (1955).

[10] A. I. Roitbak, Uchenye Zapiski LGU, Ser. Biol., 1954, No. 176 (37), 256-272.

[11] V. S. Rusinov, Physiology,* pp 235-255. Leningrad, 1954.

[12] Yu. M. Uflyand, Uchenye Zapiski LGU, Ser. Biol. Nauk, No. 123, 239-250 (1950).

[13] E. D. Adrian, Arch. Neurol. a. Psych., 1934, Vol. 32, No. 6, pp. 1125-1136.

* In Russian.

EFFECT OF STIMULATION OF RECTAL RECEPTORS OF DOGS ON THE ELECTRICAL ACTIVITY OF THE BRAIN

N. N. Lazuko

From the Department of Physiology (Director: P. S. Kupalov, Member Acad. Med. Sci. USSR), Institute of Experimental Medicine (Director: D. A. Biryukov, Corresponding Member Acad. Med. Sci. USSR), Acad. Med. Sci. USSR, Leningrad.

(Received November 14, 1955. Presented by P. S. Kupalov, Member Acad. Med. Sci. USSR)

There is very little published material on the effect of stimulating various internal organs on the electrical activity of the brain of animals and men.

F. M. Lisitsa [2], in critical experiments on rabbits and dogs, observed alterations in cortical potentials during stimulation of the stomach and rectum by distention of rubber balloons inserted therein. He found an initial raising of the amplitude of the alpha waves on the electroencephalogram (EEG), followed by a lowering of amplitude and and an increase in fequency. This author regarded the lowering of the amplitude of the alpha waves as an effect of inhibition, and he connected it with reflex cardiovascular changes.

V. E. Delov [1], showed in critical experiments on rabbits that the action of various agents on internal organs was reflected in the bioelectrical activity of definite regions of the cerebral cortex, by "initial inhibition with subsequent raising of electrical activity."

P. O Makarov [3] showed that distention of the human stomach lowered the amplitude and the frequency of the electrical activity of the cerebral cortex.

The present paper reports the results of extensive experiments designed for investigation of alterations in the biopotentials of a dog's brain due to systematic prolonged stimulation of the rectal receptors.

Our experiments were performed on one dog, which had been subjected to excision of the temporal muscles. The dog was trained to lie still on the bench in a screened room. The rectum was stimulated by means of a specially constructed attachment made of Plexiglass, 5.5 cm long and 1 cm in diameter, which caused moderate distention of the rectum lasting continuously over the 15 minutes of the experiment. Recording of the cerebral biopotentials was made from the right and left temporal regions, from silver electrodes attached to the skin with the aid of Mendeleev adhesive. Recordings were made using a cathode oscillographic equipment with a 2-channel amplifier. The range of frequencies was from 2 to 500 cps.

EXPERIMENTAL RESULTS

We first recorded the EEG for both hemispheres, in order to ascertain the normal electrical activity of the brain under the experimental environmental conditions. The normal electrograms were practically symmetrical for both sides, with respect to amplitude and frequency, with a constant alpha-like activity, manifested by waves of a frequency of about 6 per second and a potential of 25 μv, and a beta-like activity, consisting of waves of a frequency of about 38 per second and an average potential of 10.5 μv (Figure 1).

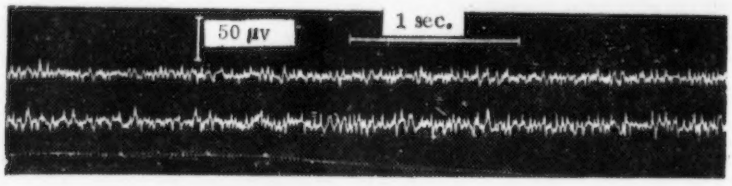


Fig. 1. EEG of the temporal region (before stimulation). Explanation of tracings (from above down): left hemisphere, right hemisphere, stimulation marker.

Having determined the initial background electrical activity, we proceeded to the systematic stimulation of the rectum, by insertion of the bougie, which caused a moderate sustained distention of the sphincter as well as of the rectum itself.

We performed 185 experiments, in all. During the first 3 days of the experiments the dog manifested some uneasiness. By the 7th day it lay quite still.

During the first 20 days of experimentation the EEG showed increase amplitude of the alpha-like activity, up to 30μv , and of the beta-like activity, with a marked preponderance of the latter. The average frequency was 50 per second, and the potential varied from 22 to 40 μv (Figure 2).

On the 30th day of experimentation slow waves began to appear from both hemispheres, with a frequency of about 4 per second and a potential of 30 to 60 μv. At the same time bursts of spike potentials appeared on the EEG, chiefly from the left hemisphere, of a frequency of 22 per second and a potential of 46 μv, on the average (Figure 3). The nature of the EEG remained steady thereafter over a month. In order to ascertain the stability of these changes, the dog was left alone for 2 weeks, after which the experiments were resumed, but without applying the stimulus.

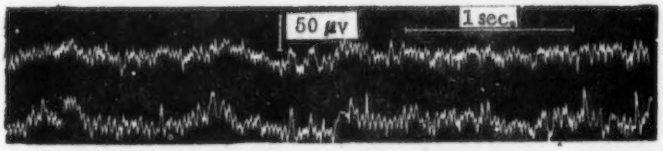


Fig. 2. EEG from the temporal region recorded during the first 20 days of experimentation involving stimulation. Intensification of fast waves, with a pronounced preponderance of beta-like activity at the moment of rectal stimulation.
Explanation of tracings as in Figure 1.

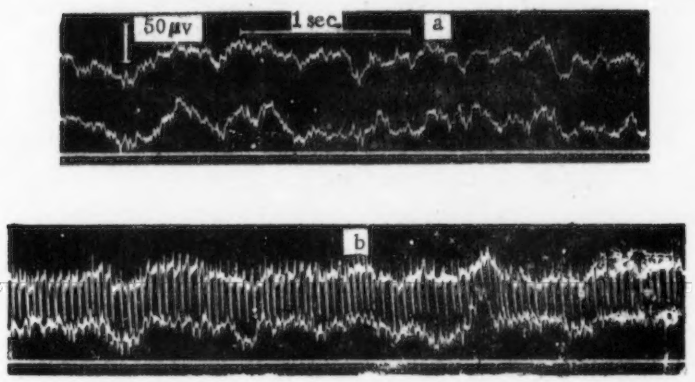


Fig. 3. EEG of the temporal region. Changes appearing on the 30th day of stimulation experiments.
a) Slow waves; b) spike potentials in the left hemisphere.
Explanation of tracings as in Figure 1.

No change was seen in the EEG for 13 days after the rest period, the same slow waves and spike-shaped oscillations being observed as before the rest period. The slow waves disappeared altogether on the 14th day. The spike potentials continued for 30 days of observation, although they occurred much less frequently towards the end of this period. Notwithstanding the rest and the cessation of stimulation, however, the EEG displayed a growing increase in the amplitude and frequency of fast rhythms, and bursts of activity of muscular origin began to appear (Figure 4).

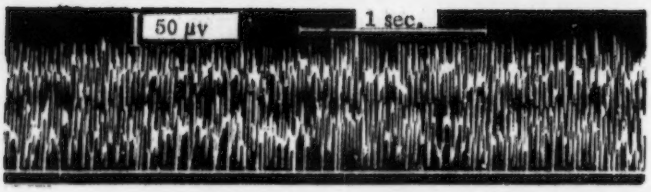


Fig. 4. EEG from the temporal region after cessation of rectal stimulation. Marked increase in amplitude and frequency of fast oscillations in both hemispheres.
Explanation of tracings as in Figure 1.

An abrupt change in the behavior of the animal was observed a month after the beginning of a new series of experiments. The dog resisted being taken to the experimental room, and tried to get off the bench when there; it sat in a corner of the dog house, and ate reluctantly. In order to exclude the possible effect of the experimental environment, which the dog would associate with the preceding experiments, we transferred it to a second screened room, where the observations were continued for a further 15 days. This did not, however, affect the nature of the EEG or the behavior of the animal.

We concluded, on the strength of the EEG changes and of the behavior of the animal, that it had developed a neurotic condition. We applied bromide treatment, in order to strengthen the inhibitory process. The electrical activity of the brain began to subside on the 3id day of treatment, and had reverted to the initial level by the 10th day, when behavior of the dog was again normal.

Our experiments show that a gradual raising of excitability of nerve centers, including those of the brain, supervenes as a result of a continuous stream of afferent impulses entering the central nervous system as a result of mild stimulation of the rectum. As a result, the excitatory process was intensified, this being reflected in the EEG by increased amplitude and frequency of fast waves of beta-like activity. The administration of bromide, which strengthens the inhibitory process, fairly quickly restored the balance between the basic nervous processes – excitation and inhibition. The persistence of the state of heightened excitability in the absence of stimulation of the rectum may most probably be explained on the basis of conditioned reflex connections with the experimental environment.

LITERATURE CITED

[1] V. E. Delov, Trudy Voenno-morskoi Meditsinskoi Akademy, 17, 117-146 (1949).

[2] F. M. Lisitsa, Byull. Eksptl. Biol. i Med., 1941, No. 12, 261-263.

[3] P. O. Makarov, Fiziol. Zhur. SSSR, 38, No. 3, 281-287 (1952).

EFFECT OF PROGESTERONE, FOLLICULIN AND CASTRATION ON THE ELECTROENCEPHALOGRAM OF FEMALE RABBITS

G F Khiustaleva

From the Laboratory for Normal and Pathol. Physiology (Director: Prof. N. L. Garmasheva), Institute of Obstetrics and Gynecology, Acad. Med. Sci. USSR (Director: Prof. P. A. Beloshapko)

(Received February 2, 1956. Presented by V. N. Chernigovsky, Member Acad. Med. Sci. USSR)

A large amount of experimental and clinical material has been published in recent years on alterations in the state of the nervous system of women during the menstrual cycle and at the menopause, i.e., at times when the amount and the relative proportions of the ovarian hormones undergo change. Thus L. I. Shvang and I. Ya. Romm (2) have reported alterations in the electroencephalogram of healthy women occurring a few days before, and during the first days of menstration.

It is now possible to examine separately the effect on the central nervous system of estrogenic hormones and of the luteal hormone, and such an examination is of importance for obtaining more precise indications for hormone therapy in gynecology.

The object of our research was to study the electrical activity of the cerebral cortex of female rabbits after administration of various doses of folliculin and progesterone, as well as after castration.

EXPERIMENTAL METHODS

Electroencephalograms were recorded from 7 female rabbits, under conditions of series experimentation. Indwelling electrodes were previously implanted into all the animals.

The electrodes, which had been provided with a screw-thread, were screwed through the cranium in the frontal and parietal regions, so that they were in contact with the meninges.

The potentials were recorded using an oscillographic equipment, with a symmetrical amplifier and with electronic registration. The records were taken on moving cinematographic film. The electroencephalograms (EEG) were evaluated visually, by comparing one with another (constant time of the amplifier T = 1.15 seconds).

We usually began the recordings on the 3rd to 4th day after implanting the electrodes, and the recordings were taken twice daily for one animal, at 9 AM and 4 PM, and three times daily for the others, 9 AM, 12 noon, and 4 PM.

Administration of sex hormones began a few days after taking the first electroencephalograms, and was repeated daily, after recording the first EEG. We injected oily solutions of progesterone (Leningrad Chemical-Pharamaceutical Factory) in doses of 1 to 20 mg per diem, and oily solutions of folliculin (Ukrainian Institute of Experimental Endocrinology) in doses of 50 to 5,000 mouse units per diem. Some animals were first given folliculin, and then progesterone, while others received progesterone alone.

EXPERIMENTAL RESULTS

The most clear cut results were given by two rabbits, in the spring-summer season.

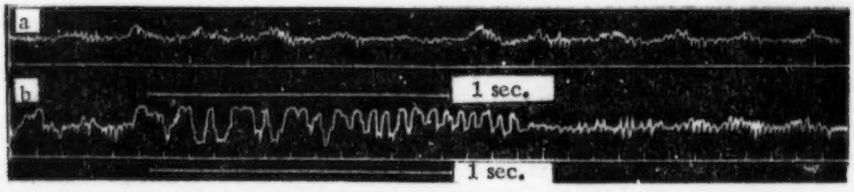


Fig. 1. Electroencephalogram of female rabbit No. 1. a) Background; b) 7 hours after injection of 1 mg of progesterone.

One of these rabbits received progesterone alone, at dosage levels of 1 mg daily for 3 days, 5 mg on the 4th, and 20 mg on the 5th day. The other doe was given folliculin: 50 International Units the first day, 200 I. U. the 2nd, 500 I. U. the 3rd, 1,000 I. U. the 4th, and 5,000 I U. on the 5th day. Progesterone was given 4 days after the last injection of folliculin, in the same doses as for the first rabbit. The animals were castrated one month after the last injection of progesterone.

The EEG of the first rabbit, recorded before administration of progesterone, shows the presence of slow waves, of a frequency of 3-4 per second, and of fast waves, frequency 60-65 per second (Figure 1, a).

The first two injections of progesterone did not bring about any visible changes in the EEG.

Seven hours after the third injection of 1 mg of progesterone the EEG showed, in addition to the slow waves of frequency 3-4 per sec., and to the fast waves, frequency 60-65 per sec., sections of the tracing consisting of waves of a frequency of 10-15 per sec., of varying amplitude (Figure 1, b). These changes were no longer in evidence by the morning, the EEG being of the normal type. Similar slow waves of frequency 10-15 per sec. were again seen 7 hours after injecting 5 and 20 mg of progesterone. With increase in the dose of progesterone the number of sections showing these waves increased. These changes again disappeared by the next morning after the injections. After injecting 20 mg of progesterone behavioral changes also appeared in the animal: it refused food, avoided the other rabbits, and became much less active.

The EEG of the second rabbit, recorded before injection of hormones, shows the presence of slow waves, frequency 2-3 per sec., and fast waves, frequency 50-55 per sec (Figure 2, a).

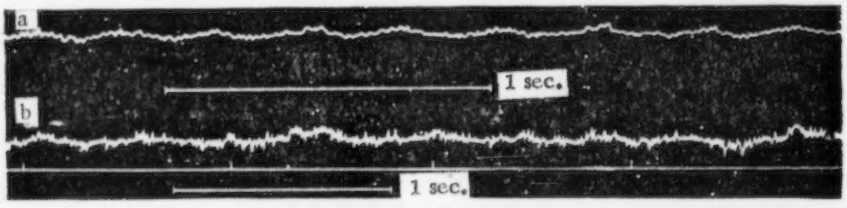


Fig. 2. Electroencephalogram of female rabbit No. 2. a) Background; b) 7 hours after injection of 500 I. U. of folliculin.

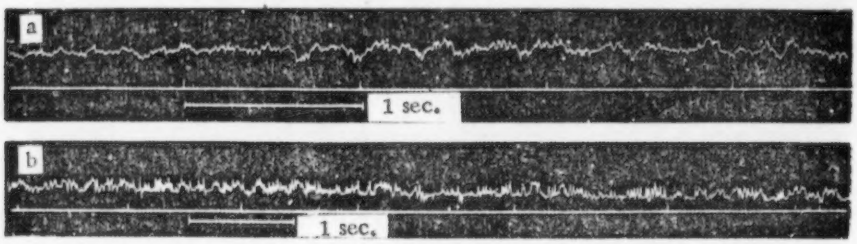


Fig. 3. Electroencephalogram of female rabbit No. 2. a) Before castration (1 month after the last progesterone injection); b) 12 days after castration.

Injection of 50 and 200 I. U. of folliculin on two successive days did not cause any evident changes in the EEG. Three hours after injection of 500 I. U. of folliculin there was still no change in the EEG, but 7 hours after injection of 500 I. U. of folliculin it was possible to observe an increase in the frequency of the fast waves, to 60-65 per sec. (Fig. 2, b). This effect was still in evidence on the following morning.

Injection of 1,000 I. U. of folliculin reduced the frequency of the fast waves to 50 per sec., and injection of 5,000 I. U. of folliculin on the following morning caused no further change in the EEG.

The EEG recorded 4 days after the last folliculin injection showed slow waves, frequency 2-3 per sec., and fast waves, frequency 52-55 per sec.

The first two injections of progesterone had no perceptible effect on the EEG. Three hours after the third 1 mg injection the EEG showed slow waves, frequency 10-15 per sec. The changes were similar to those seen after the third injection of progesterone into the previous rabbit.

Stretches of slow waves of frequency 10-15 per sec.could not be seen on the EEG until 48 hours after the 4th injection of 5 mg of progesterone, when this type of wave predominated.

The slow waves (10-15 cycles/sec.) were seen on the EEG recorded at 3 and 7 hours after injection of 20 mg of progesterone. The slow wave activity disappeared from the EEG as soon as the injections were discontinued. One month after the last injection of progesterone the EEG showed slow waves of a frequency of 3 per sec., and fast waves of a frequency of 50-55 per sec., i.e., the EEG had reverted to the initial pattern.

Castration was then performed, and this was followed by pronounced changes in the behavior of the rabbit.

Twelve days after castration clear-cut changes were evident in the EEG: the slow waves were absent, and waves of a frequency of 50-52 per sec. predominated, with a diversified amplitude (Figure 3, b).

Examination of the EEG for the two rabbits showed that the amplitudes of the waves in the morning recordings were smaller than for the evening ones.

The effects of folliculin and progesterone on the EEG of the other rabbits, which were recorded during the winter season, were much smaller, but in some of the experiments we found that administration of progesterone was followed by the appearance of slow waves of the same type as for the first two animals. The effect of folliculin was barely perceptible.

It thus appears that progesterone, whether administered after folliculin or before, caused the appearance of slow waves of a characteristic frequency. These waves appeared in two cases after administering 3 mg of progesterone over 3 days. The incidence of these slow waves increased with increasing doses of progesterone, and with diminution in the motor activities of the animals.

Doses of 750 I. U. of folliculin administered over 3 days caused an increase in the frequency of the waves. Further increase in the folliculin dosage to 5,000 I. U., led to a fall in the frequency, which did not, however, fall below the initial value.

Castration was followed by behavioral changes in the animals, and by disappearance of the slow waves, while the amplitude of the fast waves fluctuated widely throughout the recording.

Our experimental findings show that progesterone and folliculin exert different actions on the functional state of the cerebral cortex.

LITERATURE CITED

[1] M. K. Petrova, Trudy Fiziol Lab. Akademika I. P. Pavlova, Vol. 6, No. 1, 5-115 (1936).

[2] L. I. Shvang and I. Ya. Romm, Akusher. i Ginekol., 1953, No. 1, 25-32.

EFFECT OF STIMULATION OF THE HYPOTHALAMIC REGION ON REFLEX FLUCTUATIONS OF SKIN POTENTIALS OF FROGS

I. V. Vvedenskaya

From the Electrophysiology Laboratory (Director: Prof. A. V. Lebedinsky, Corresponding Member Acad Med. Sci. USSR), Leningrad Scientific Research Neurosurgical Institute (Director: Prof. V. N. Shamov, Member Acad. Med. Sci. USSR)

(Received September 29, 1955. Presented by V. N. Shamov, Member Acad. Med. Sci. USSR)

Most authors, in considering the problem of Sechenov-type inhibition, are of the opinion that there is no need to postulate the existence of special inhibitory structures in the brain, in order to explain development of central inhibition. The basic task of research workers is to elucidate the intimate mechanisms of development of inhibition in the spinal cord, in relation to stimulation of the brain. Developing the view expressed by Academician L. A. Orbeli [2, 3], on the adaptive-trophic function of the sympathetic nervous system, A. V. Tonkikh [7, 8] elucidated experimentally the phenomenon discovered by I. M. Sechenov [4] in 1862, as being an effect emerging from the diencephalic vegetative centers, and mediated through the sympathetic system.

In the classical form of Sechenov's experiment this action of the sympathetic system is manifested by the alteration in the course of the motor reflex, and practically all those who have studied this phenomenon have done so on the basis of the motor reactions [1, 5, 6, 8, 9].

The object of the present research was to investigate the nature of the action of the diencephalon on vegetative reflexes, using Sechenov's method of stimulating the hypothalamic region. In addition, we hoped, by comparing these findings with the known effects of visual processes on motor reflexes, to gain some insight into the action of vegetative centers on both groups of reflex reactions. As an indicator of vegetative reactions we selected the reflex changes in skin potentials (emf) occurring when it is subjected to chemical (acid) stimulation. These effects were correlated in some of the experiments with the motor reactions taking place on the application of acid.

EXPERIMENTAL METHODS

Our experiments were performed on frogs. We measured the changes in skin potential by a compensatory method. A potentiometer and a mirror galvanometer were connected in circuit with the parts studied. The magnitude of the skin potentials and of their fluctuations were measured in millivolts. The skin potentials were taken off from the hind legs of the frogs, which were immersed up to the knee in physiological saline (0.65% NaCl). One nonpolarizing agar-agar electrode was placed in the vessel containing saline, and the other was connected through a cotton pad soaked in saline to the exposed, but undamaged, dorsal muscles of the frog. In this way we could lead the current off from the inner and outer surfaces of the skin. Chemical irritation of the skin was performed by placing a piece of filter paper of area 0.25 sq. cm , wetted with 1% sulfuric acid, on the skin of the thigh.

One of the objectives of our experiments was to elucidate the effect of stimulating the diencephalon of the frog on the nature of vegetative reflexes.

The experiments were performed in the following way. The movements of the animal were slightly restricted by giving appropriate doses of curare, the skull was opened, and the brain was transected at the level of the anterior boundary of the mesencephalon, as was done by I. M. Sechenov in his experiments. After an interval of an hour, the frog was switched into the potentiometer circuit. Having established a more or less definite background level of reflex variations in the skin potential (stimulation of the skin was applied for 30-second periods, at 5-minute intervals), we placed a sodium chloride crystal on the mesencephalon, leaving it there for 1 minute, during the last 15 seconds of which we also applied skin stimulation. This permitted of the registration of alterations in reflex changes in skin potentials, proceeding against a background of stimulation of the mesencephalon. We used 100 frogs in our experiments.

EXPERIMENTAL RESULTS

In most cases we found heightening of reflex fluctuations in skin potentials during periods of stimulation of the mesencephalon (Table 1).

The enhancement of excitability of the reflex apparatus of the spinal cord, manifested as increase in reflex skin potential oscillations, was particularly well marked in those cases where the reflexes were of low strength. At times, they disappeared altogether during an experiment, and only reappeared after preliminary stimulation of the mesencephalon. An effect of this sort was recorded by taking a photographic tracing of the movements of the galvanometer mirror (see Figure).

Apart from these effects, we observed cases in which stimulation of the mesencephalon led to a lowered excitability of the reflex apparatus, manifested by diminution in amplitude of the reflex changes in skin potential, and by a shortening of the latent period. In a number of the experiments reflex variations in skin potential disappeared completely during stimulation of the mesencephalon, as is evident from Table 2.

TABLE 1

Time		Reflex potential changes, mv
hours	minutes	
12	00	10
12	05	5
		Stimulation of the mesencephalon
12	10	15
12	15	8
12	20	5
		Stimulation of the mesencephalon
12	25	6
12	30	3
12	35	2
		Stimulation of the mesencephalon
12	40	4,5
12	45	4
12	50	4
		Stimulation of the mesencephalon
13	00	5
13	05	5
13	10	2

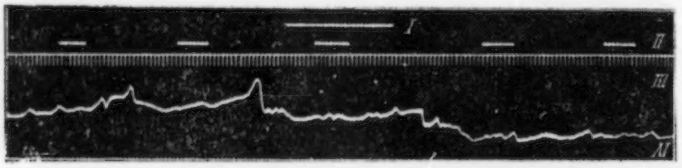
Thus our results are evidence of the adaptive-trophic action of central sympathetic nerve elements of the mesencephalon on vegetative reflexes.

TABLE 2

Time		Reflex potential changes, mv
hours	minutes	
11	30	5
		Stimulation of the mesencephalon
11	35	0
11	40	4
		Stimulation of the mesencephalon
11	45	0
11	50	3
		Stimulation of the mesencephalon
11	55	0
12	00	3

We compared our results on the effect of the mesencephalon on vegetative reflexes with the known effects of visual centers on motor reflexes. The experiments were designed to permit of the simultaneous observation of

motor and autonomic system reactions. The latent period found for the motor reflex was much shorter than that of the vegetative reflex, in response to one and the same stimulus. We found, further, that in accordance with A. V. Tonkikh's findings, the latent period of the motor reaction was lengthened during stimulation of the mesencephalon, whereas we often observed shortening of the reflex reaction time for the vegetative reaction, with strengthening of the reflex itself. In some cases, the changes in both types of reaction were in the same direction during stimulation of the mesencephalon. Together with lengthening of the latent period of the motor reaction we found a lengthening of the latent period of the vegetative reaction, up to its total abolition.



Increase in reflex skin potential variations observed during stimulation of the mesencephalon with a crystal of salt.
I) Signal indicating time and duration of stimulation of the mesencephalon with a salt crystal; II) signal showing time and duration of stimulation of the skin with sulfuric acid; III) time marker; IV) photorecording of movements of the light spot of the galvanometer.

We were also able to establish that the upper boundary of emergence of sympathetic fibers responsible for skin-potential variations, and effecting communication between the spinal cord and the higher levels of the central nervous system, are at the level of the 2nd segment of the spinal cord.

LITERATURE CITED

[1] N. A. Adamovich et al., Trudy Inst., Mozga im. Bekhtereva, pp. 49-50 Leningrad, 1947.

[2] L. A. Orbeli, Fiziol. Zhur. SSSR, 15, No. 1-2, 1-22 (1932).

[3] L. A. Orbeli, Vrach. Gaz., 1927, No. 3, 163-169.

[4] I. M. Sechenov, Selected Works, Moscow, 1935.

[5] N. A. Soloveva, Papers Read at the Scientific Workers' Congress, Held to Celebrate the 18th Congress of the All-Union Communist Party, pp. 40-41, Leningrad, 1939.

[6] B. D. Stefantsev, ibid, pp. 43-44.

[7] A. V. Tonkikh, Fiziol. Zhur. SSSR, 8, No. 576, 31-50 (1925).

[8] A. V. Tonkikh, Fiziol. Zhur. SSSR, 13, No. 1, 11-18 (1930).

[9] V. A. Cherkes, Voprosy Fiziology, Vol. 8, pp. 13-33 Kiev, 1954.

• In Russian.

DETERMINATION OF EXCITABILITY OF HUMAN MUSCLES

D. L. Rozental and V. N. Filippova

From the Research Group working with Prof. D. N. Nasonov, Member Acad. Med. Sci. USSR, at the Leningrad Scientific Research Institute of Traumatology and Orthopedics (Scientific Director: Prof. S. S. Girgolav, Member Acad. Med. Sci. USSR)

(Received November 10, 1954. Presented by D. N. Nasonov, Member Acad. Med. Sci. USSR)

It has been shown by D. N. Nasonov and D. L. Rozental (3) that chronaxie "is not a measure of the speed of a tissue reaction, or of the excitability of the given tissue." For the characterization of excitability, the use of the constants b, the intensity excitability threshold (rheobase), and a, the duration threshold of excitability, was proposed. These constants may be derived from the formula

$$v = \frac{a}{t^n} + b,$$

where v is the threshold potential, in volts, and t is the time of action of the current, in milliseconds (msec).

If t^n is large enough, $v = b$. If b is small enough, then $v = a/t^n$, when a will be "the threshold potential which causes stimulation within a given time of action, chosen by us as a unit, for sufficiently short intervals." Taking v in the formula $v = a/t^n$ (for $n = 1$) as unity, a will define the duration of the stimulation. Chronaxie will in no way define this time, since it follows from the formula $v = a/t^n + b$, when $v = 2b$, that

$$2b = \frac{a}{Chr} + b$$

and hence that

$$b = \frac{a}{Chr}, \quad \text{or} \quad Chr = \frac{a}{b},$$

i.e., chronaxie will depend on the ratio of a to b, and these may vary relatively independently of each other. On the strength-duration curve chronaxie defines the point of inflection, where one expression, dependent on time, is replaced by another, time-independent one.

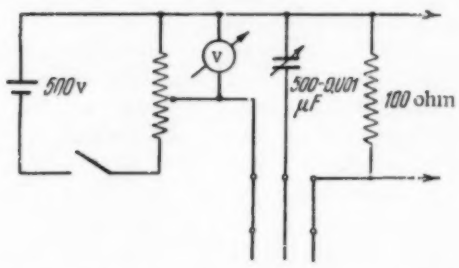


Fig. 1. Circuit diagram for determining excitability.

Inasmuch as chronaxie expresses some property of tissues, the possibility remains to be investigated that, although it is not a measure of excitability of tissues, it might still serve some clinical purpose, possibly a diagnostic one. The chronaximetric method is widely applied clinically, since chronaxie is regarded as a parameter characteristic of the speed of development of excitation. No independent significance is attributed to the rheobase, which serves only for measurement of chronaxie. In their reviews of chronaximetry, D. A. Markov [1] and Yu. M. Uflyand [6] frequently present data for chronaxie, without giving the corresponding rheobase values.

We have examined the dynamics of short- and long-term excitability of normal and pathological human muscles, as also their chronaxie. In our investigation we used a modified chronaximetric method, in which the Lapicque shunt was replaced by a 100 ohm shunt [3]. The circuit is represented in Figure 1.

We did not, as was recommended by D. N. Nasonov [2], include a resistance in series of 50-100 ohms, since this is in effect replaced by the resistance of the tissues through which the current passes to the nerve. The

thresholds of excitability of the nerves and muscles of the intact organism are higher than those of the isolated tissues, but because of the 100 ohm shunt we were able, using a current of 400-500 v, to obtain the data for a full strength-duration curve for the intact organism.

Most modern condenser chronaximeters used for determination of rheobase make use of d.c. impulses. The subject of such measurements usually experiences painful sensations (6). In our experiments, determination of the rheobase was painless, using condenser discharges of considerable duration.* It is the usual practice in chronaximetry to apply the electrode to the motor point for each measurement of excitability. We think that this procedure suffers from a number of disadvantages. A displacement of a few millimeters of the electrode from the motor point, or its firmer application, lead to quite different results of excitability measurements. Tying the electrodes down, as is sometimes practiced, is not to be recommended, since the electrode readily undergoes displacement under such circumstances [1]. The degree of moistening of the electrode is also of importance. It is not surprising that only experienced operators are able to achieve reproducible results.

For these reasons we modified the usual method, for the study of the dynamics of excitability over short periods, not exceeding a few days.

As a measuring electrode we used a chlorinated silver cup, area 1 sq. cm, and depth 4 mm. The cup was filled with cotton-wool impregnated with 2% agar made up with physiological saline. Having found the motor point, we attached the electrode by means of sticking plaster. This modification assured a constant moisture level, constant pressure of the electrode against the skin, and firm fixation to the motor point. Errors are thus largely eliminated, although displacement of the motor point may still take place if the position of the arm is changed.

We determined the strength-duration curves in each experiment, which could very conveniently be achieved with the attached electrode. If we had applied the electrode separately for each reading we should either have had to find the point of minimum excitability anew for each capacity, or to apply the electrode each time to a previously marked motor point. In the former case, we would have had to devote very much time to the preparation of the curves, while in the latter case, in addition to all the usual shortcomings inherent in this procedure, we would still be left with the possibility of errors due to shifting of the motor point.

Having once constructed the whole of the strength-duration curve, we could thereafter confine ourselves to the measurement of the constants a and b. The value of the constant a was obtained in the following way: we selected the threshold potential for a sufficently short time interval (the maximum potential and the smallest capacity achievable with our equipment). Since it follows from Horweg's formula that when $n = 1$, $a = (v - b)t$, then from the threshold potential found by us we could derive the value of the rheobase and of the difference $(v - b)$, expressed in mv, and this multiplied by time, in msec., gave the value of a, expressed in millivolt-milliseconds. Determination of the rheobase and of the constant a takes no more time than is needed for measurement of chronaxie.**

It is possible to derive the value of a from the formula $(v - b)t$, or from vt, only in the case when $n = 1$; in other cases, t^n has to be taken. We found that $n = 1$, or is very close to 1, for the strength-duration curves of human arm muscles. A logarithmic curve of this sort is shown in Figure 2. This also gives the values of the constants a and b, and of chronaxie, and n is calculated as $\tan\alpha$. In order to derive chronaxie from the curve, a distance of log 2 is marked off on the ordinate axis, above the point corresponding with unit b, and a horizontal line is drawn parallel to the abscissa axis, to the point of intersection with the curve. A perpendicular dropped from this point to the abscissa axis gives the value of log Chr.

---

* According to L. K. Rubin [5] it is inadmissible to determine chronaxie and rheobase using impulses of current of different forms, as it would not be possible to know whether chronaxie was determined for the same fibers as those for which rheobase was determined, or for different ones. This is a further argument supporting the view that rheobase should be determined using condenser discharges of prolonged duration.

** The value of the rheobase is not taken into account in measuring the value of a for frog nerve muscle preparations, since it is much smaller than the threshold potential for currents of short duration. In this case a is given by the product of v and t [4].

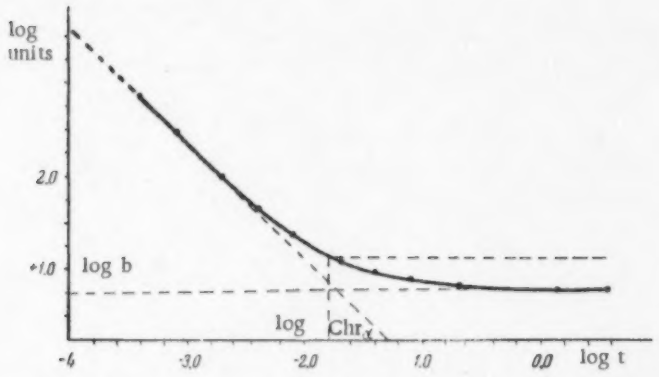


Fig. 2. Logarithmic strength-duration curve for the patient F.
n = 1; a = 118 mV in 1 millisecond; b = 6.6 v ; Chr = 0.0159 msec.

The majority of the experimental strength-duration curves found by us for human arm muscles are in fairly close agreement with the Horweg-Weiss formula. This is clearly evident in Table 1, which gives the deviations of the experimental from the calculated results. Since n = 1 for all the curves of Table 1, we could apply the formulae $v = a/t + b$ or $a = (v-b)t$. The numerical values of threshold potential for the biceps muscle of the patient F are given in Table 1 (calculated values v_1, and experimental values v_2).* For the other patients we give only the differences between the calculated and experimental values (expressed as percentages of the calculated values). In all cases, the deviations are so small that we are justified in assuming that the experimental curves for human muscles correspond with those calculated from the Horweg formula, i.e., with the theoretical curves. It should be noted that the deviations of experimental curves for frog nerve-muscle preparations from the theoretical ones are considerably greater, especially around the inflection point of the curve.

TABLE 1

Threshold Potentials for the Biceps Muscle, Found Experimentally and Calculated from the Formula $v = a/t + b$.

	Patient F., 10/10;			Patient A	Patient R	Patient V	Patient A	
							10/27	11/10
	v_1 calculated from the formula	v_2 experimental*	$\frac{v_1 - v_1}{v_1}$	$\frac{v_2 - v_1}{v_1}$	$\frac{v_2 - v_1}{v_1}$	$\frac{v_2 - v_1}{v_1}$	$\frac{v_2 - v_1}{v_1}$	$\frac{v_2 - v_1}{v_1}$
			in %					
90	—	6.6	—	—	—	—	—	—
30	—	6.6	—	—	—	—	—	—
5	7.17	7.2	+0.4	+ 6.3	— 0.3	+4.4	+ 4.3	— 3.86
2	8.0	8.2	+2.5	+ 2.28	— 1.58	+1.95	+ 2.6	+ 1.54
1	9.47	9.4	—0.074	+ 2.2	+ 1.77	+3.6	+ 6.1	+ 2.6
0.5	12.35	11.2	—0.93	+ 7.8	—13.3	+0.62	+ 7.5	— 9.7
0.2	21.0	20	—5	+ 8.1	— 6.25	0	— 8.0	— 3.1
0.1	35.3	34	—3.7	— 0.26	— 3.62	—9.8	— 2.0	— 2.7
0.05	64	62	—3.12	— 2.16	— 7.25	—2.6	— 2.2	— 8.4
0.02	150	150	0	+ 2.16	+ 7.2	0	+ 2.9	— 9.2
0.01	293.6	310	+5.6	— 0.87	— 8.2	—8.6	0	+ 9.4
0.005	—	—	—	+12.1	—	—	—12.9	—16.8

* The numerical values contained in this column were taken for the construction of the strength-duration curve of Figure 2.

In our studies of pathologically altered muscles we examined leg muscles chiefly; their excitability thresholds were higher than those of the upper extremities, probably owing to the greater resistance of the intervening skin layer. For this reason, we could not get enough points on the strength-duration curves for the accurate determination of n. We believe, however, that the slope of the strength-duration curves for leg muscles is the same as for arm muscles, i.e., that $n \approx 1$.

A comparison of the excitability of normal and pathologically changed muscles (this is possible only on the condition that there is no great difference between the resistance of the two extremities) shows that the strength-duration curves for diseased muscles lie above those for normal ones (Figure 3). This means that both the intensity and the duration thresholds have been raised. The chronaxie changes are in most cases in the same direction

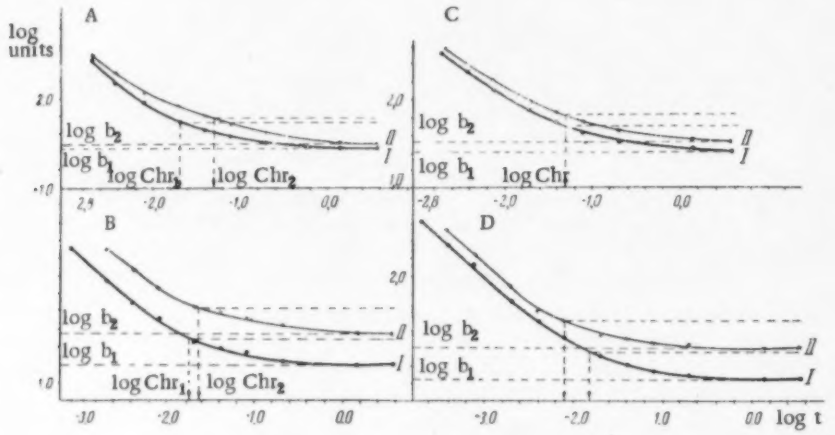


Fig. 3. Logarithmic strength-duration curves for normal and pathological muscles.
A) Patient S. Ankylosis of the right knee-joint; Rectus femoris muscle: .–.–.–healthy leg: a=503 mv in 1 msec.; b = 28v; Chr = 0.0209 msec.; x–x– diseased leg: a= 580 mv in 1 msec.; b = 30v; Chr = 0.0502 msec. B) Patient A. Sequelae of osteomyelitis: Rectus femoris muscle: .–.–.–healthy leg: a= 248 mv in 1 msec.; b = 16 v; Chr = 0.0174 msec.; x–x–diseased leg: a = 532 mv in 1 msec.; b = 36v; Chr = 0.0219 msec. C) Patient S. Ankylosis of the right knee-joint; Muscles of the semi-group: .–.–.–healthy leg: a = 592 mv in 1 sec.; b = 24 v; Chr = 0.68 msec.; x–x–diseased leg: a = 696 mv in 1 sec.; b = 24v; Chr = 0.61 msec. D) Patient G. Residual symptoms of spastic paralysis of the left arm: .–.–.–healthy arm: a = 80mv in 1 sec.; b = 7v; Chr = 0.016 msec.; x–x–diseased arm: a = 127 mv in 1 sec.; b = 16 v; Chr = 0.008 msec.

as are the changes in the strength-duration curves, i.e., the lowering of excitability of the pathologically changed muscles is associated with prolongation of chronaxie. This correlation is, however, basically a qualitative one. We very frequently encountered a large increase in chronaxie together with a small rise in excitability threshold (in Figure 3A, b is increased by 7%, and a by 14%, while chronaxie is increased by 150%). * Figure 3B illustrates another not infrequently encountered case, in which there is a considerable, though uniform rise in excitability threshold (b by 111%, and a by 112%), with only a small increase in chronaxie (by 20%). There are cases in which chronaxie gives a distorted picture of the functional state of the muscles. Two such cases are represented in Figures 3C and 3D. Figure 3C shows the strength-duration curves for the semitendinosus and semimembranosus group of muscles of the diseased leg (ankylosis of the knee joint) of the patient S.

* Here the increase in chronaxie is due to the deviation from the calculated values curve in its middle part.

There is a marked fall in excitability (with a corresponding prolongation of reaction time), but in all the points of the strength–duration curve for the diseased leg the chronaxie of the muscles is the same as for the healthy leg. From chronaxie data alone we should have come to the conclusion that the functional states of the healthy and the diseased muscles are identical.

An even more instructive example is given by Figure 3D. The upper curve, for patholgically changed muscle is considerably displaced from that for normal muscle. The duration excitability threshold is raised from 130 v for the healthy leg to 170 v for the diseased one, and the rheobase from 7 to 16 v, with considerable shortening of chronaxie, from 0.016 to 0.008 msec. The reason for this was that the rheobase had risen more than the duration excitability threshold; an inexperienced observer might have drawn the erroneous and somewhat paradoxical conclusion that the diseased muscles were in a good functional condition.

Although such cases may be seldom encountered, their possibility is a sufficient objection to using chronaxie measurements for diagnostic purposes. We have not encountered any such discrepancy between the clinical picture and the values of the constants $\underline{a}$ and $\underline{b}$. In all the cases presented by us, the curves for pathologically changed muscles showed higher duration and intensity excitability thresholds. We found no exceptions to this among the 14 patients whom we examined.

We were able, by following changes in the values of the constants $\underline{a}$, $\underline{b}$, and chronaxie, to detect postoperational changes in excitability. We shall here give one such example. Transplantation of the femoral extensor muscles (biceps and semitendinosus) was performed in the patient G. to the lower part of the femur (for treatment of postpoliomyelitis paralysis). Table 2 presents the tabulated data for duration (a) and intensity (b) excitability thresholds, and for chronaxie, for the semitendinosus muscle before operation, and 2 months after operation, when the plaster cast was removed.

TABLE 2

Values of the Constants $\underline{a}$ and $\underline{b}$, and of Chronaxie, for the Semitendinosus Muscle of the Healthy and Diseased Legs of the Patient G., Before and After Operation

Constants	Healthy leg			Diseased leg		
	before operation	after operation	percentage change, compared with the calculated pre-operational value	before operation	after operation	percentage change, compared with the calculated pre-operational value
b (v)	28	15.6	−44.2	30	17.1	−43
a (mv in 1 msec.)	541	320	−40.7	470	504	+ 7
Chronaxie (msec.)	0.033	0.32	−3	0.024	0.05	+108

We observed considerable lowering of the intensity threshold (by 43%) for the transplanted muscle, while the duration threshold remained practically unchanged (a rise of 7% is too small to be considered as significant). At the same time, chronaxie was doubled. We know from the case history that the transplanted muscles functioned well after two months, and that the patient walked better after the operation than before. The increase in chronaxie seen in this case did not reflect change in the functional condition, but was due to diminution in the term $\underline{b}$ of the fraction a/b, while $\underline{a}$ remained without change. Excitability changes were also evident in the healthy leg, in the form of a considerable fall in intensity (by 44.2%) and duration (by 40.8%) thresholds of excitability. Since the values of the constants $\underline{a}$ and $\underline{b}$ fell parallel, chronaxie was unchanged in this case. Had we confined our examination only to chronaxie measurements we would not have perceived any changes in the functional state of the muscles.

The data presented in this paper, on changes in excitability of normal and diseased muscles, can only be regarded as being of the nature of a preliminary communication. It is necessary to accumulate more factual material, and to relate it more closely to the clinical data. We can, however, on the strength of the examples quoted, state that chronaxie can give a misleading impression of the functional state of muscles, and that a much more trustworthy picture of the functional state is afforded by intensity and duration excitability threshold data.

LITERATURE CITED

[1] D. A. Markov, Clinical Chronaximetry,* 1935.

[2] D. N. Nasonov, Fiziol. Zhur. SSSR, 41, No. 43, 554-567 (1955).

[3] D. N. Nasonov and D. L. Rozental , Fiziol. Zhur. SSSR, 39, 405-422 (1953).

[4] D. N. Nasonov and D. L. Rozental , Fiziol. Zhur. SSSR, 41, 121-131 (1955).

[5] L. K. Rubin, Nevropatol. i Psikhiatriya, 6, No. 1, 102-104 (1937).

[6] Yu. M. Uflyand, Theory and Practice of Chronaximetry,* Leningrad, 1941.

EXCITABILITY OF HUMAN MUSCLES DURING SLEEP AND WAKEFULNESS

D. L. Rozental and V. N. Filippova

From the Research Group working with Prof. D. N. Nasonov, Member Acad. Med. Sci. USSR, at the Leningrad Scientific Research Institute of Traumatology and Orthopedics (Scientific Director: Prof. S. S. Girgolav, Member Acad. Med. Sci. USSR)

(Received April 25, 1955. Presented by S. S. Girgolav, Member Acad. Med. Sci. USSR)

L. Lapicque [5] has developed the concept of the subordinative effect of centers, based on the observed increase in excitability and prolongation of chronaxie following transection of a nerve. It has, however, been shown by Nasonov and Rozental' [8] that these changes in excitability are due to impulses proceeding from the point of transection of the nerve. The apparent paradoxical increase in chronaxie, with heightening of excitability over the whole of the strength–duration curve, was explained as resulting from the unequal diminutions in the duration (constant b) and intensity (constant a) thresholds of excitability.

The changes in peripheral excitability observed by a number of workers to take place during sleep have been ascribed to disturbances in the subordinative influences of the centers on the periphery. G. Bourguignon and J. B. Haldane [14] have reported prolongation of chronaxie when the subjects fall asleep. This observation was confirmed by P. A. Kiselev and F. P. Maiorov [2, 3]. They examined a number of narcoleptic patients and of healthy individuals during sleep, and found that chronaxie is prolonged during sleep.

In their further researches, F. P. Maiorov and his co-workers studied chronaxie changes during sleep due to various causes, viz., during alcoholic intoxication [1], in aged individuals [11], in juveniles [4], hypnotic sleep [6, 12], and in narcoleptic states [9, 10].

Researchers have shown that increase in chronaxie takes place during sleep (to an extent depending on the depth of sleep), together with regular changes in the ratio of flexor and extensor chronaxies. These findings, which are of great importance for the study of sleep and of subordination, were based exclusively on the use of chronaximetric methods.

Our investigations of the effect of severing a nerve on its excitability [8] have led us to take up a critical attitude towards current concepts of subordination, although we do not reject the existence of central influences at the periphery. We therefore thought it worth-while to reexamine the above-cited data on the subordinative effects of the centers on the periphery during sleep, applying the new methods put forward by D. N. Nasonov and D. L. Rozental [7] for the evaluation of excitability.

EXPERIMENTAL METHODS

We examined peripheral excitability during wakefulness, during sleep during the day, both natural and reinforced by hypnotics, and during nocturnal sleep. We recorded strength–duration curves for the biceps brachii and flexor digitorum sublimis muscles, using a slightly modified condenser chronaximeter, in which the Lapicque shunt had been replaced by a 100 ohm shunt [7]. This permitted the recording of strength–duration curves of

*In Russian.

human muscles using currents of fairly short duration. The stimulating electrode was a chlorinated silver cup filled with 3% agar made up with Ringer solution, which was attached by strips of plaster to the skin over the motor point. This assured the firm attachment of the electrode, with uniform conditions of moisture and pressure against the skin, not depending on the judgement of the experimenter.

Excitability during wakefulness was studied in 5 persons: F., aged 37, A., aged 34, R., aged 36, V., aged 51, and K., aged 21.

A., V., and K. were also examined during sleep. The subjects were examined in a recumbent position. The variations in excitability were recorded for one and the same point daily for a month, and at 10-20 minute intervals over a few hours.* Changes in excitability were evaluated from the variations in intensity threshold (rheobase) and duration threshold (constant a). In addition, and at least once during each examination, we recorded the full strength–duration curve. Rheobase was measured in volts. The value of the constant a was derived in mv-msec. For this purpose, the threshold potential, expressed in mv, was multiplied by duration of action of the current (for short durations), expressed in msec. We derived chronaxie from the ratio a/b. This way of calculating chronaxie is admissible, in view of the circumstance that most of the strength–duration curves for human muscles have n = 1, thus closely enough satisfying Horweg's formula.

EXPERIMENTAL RESULTS

We found relatively only small variations between the values for excitability in the waking state. The maximum deviations from 100% found over a whole month amounted to +48% and -7% for duration threshold, and to +24% and -16% for intensity threshold of excitability.

Excitability varied very little during the day. The variations did not exceed ±20% of the initial excitability.

Most of the work on the effect of natural sleep on chronaxie was done on the patient A. (15 experiments). She fell asleep very readily during the day, under laboratory conditions. She slept well; her breathing was regular, and her sleep lasted for 2-3 hours. On two occasions sleep was reinforced by administration of 0.2% sodium amytal, when it lasted for 4-5 hours.

At full strength–duration curve was recorded before sleep. Thereafter, at definite intervals, we measured only rheobase and the constant a. On one or two occasions we also recorded the full strength–duration curve during sleep. This aroused no unpleasant sensations in the subject, and so could be effected even during quite light sleep.

In eleven experiments we determined the excitability of the biceps brachii muscle, and in four experiments we used the flexor digitorum sublimis.

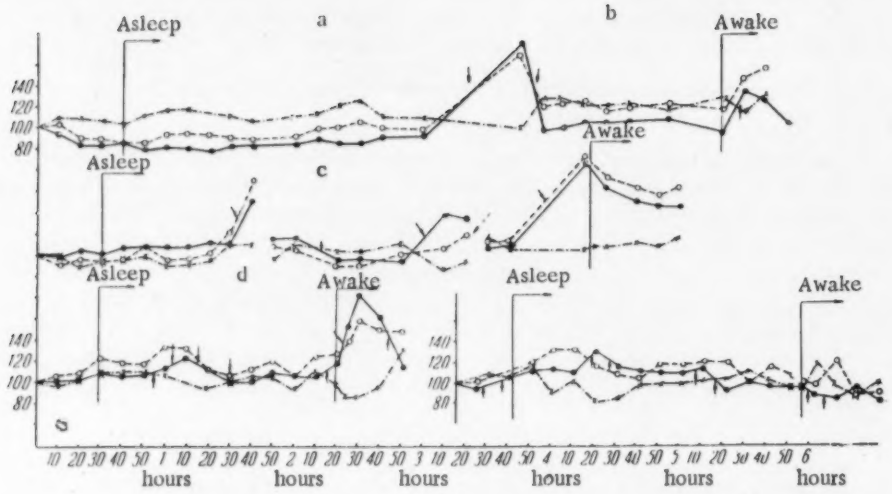
The Figure illustrates variations in b, a, and chronaxie for these muscles during daytime sleep of the subject A.

An examination of the changes found for excitability shows that shifts in the values of both intensity and duration thresholds occurred at the moment when the subject shifted the position of her arm (such moments are indicated by arrows in the diagrams). The variations in excitability observed on such occasions were probably due to displacement of the skin, and hence of the electrode, with reference to the motor point. If, after such a shift, we again found the motor point (shifting the position of the electrode by a few mm usually suffices for this purpose), excitability reverted to its initial value. Occasions on which we thus relocated the motor point are indicated in the Figure by a broken arrow. Such shifts in excitability associated with arm movements are particularly well-defined for recordings from the biceps brachii muscle. This is what would be expected, since displacement of the skin relative to the muscle takes place more readily with respect to the biceps than to the flexor digitorum sublimis.

The values of the constants a and b and of chronaxie may rise or fall during sleep, as during wakefulness. This is evidence that the variations are not associated with falling asleep or awakening, but with displacements of the electrode over the "motor point," due to movements of the arm.

---

* In observations extending over a month the electrode was removed after each daily determination, the motor point was outlined in ink, and the electrode was reattached to the same point the next morning.



Percentage changes in the values of the constants a and b and of chronaxie during daytime sleep of the subject A.
Explanation of curves: .–.–. b; x–x Chr; o–o a; solid arrows: changes in position of the arm; broken arrows: relocation of the motor point; a, b, c) biceps brachii muscle; d) flexor digitorum sublimis muscle.

In distinction to the findings of Maiorov and his co-workers, we were unable to observe any pronounced changes in chronaxie. It is evident from the Figure that the variations in chronaxie during sleep are much smaller than are those of the rheobase and of the constant a.

The results of our experiments on the subject A. (biceps muscle) are presented in the Table. This shows the variations in the values of the constants a and b of chronaxie on falling asleep and on awakening. The variations associated with falling asleep are expressed as percentages of excitability when awake, and the changes seen on awakening as percentages of the excitability found before waking. Only those experiments in which the position of the arm did not change during the recording are included in the Table. The data of this Table therefore, refer only to changes in excitability not due to displacement of the electrodes. As appears from the Table, the threefold quadratic error is so much greater than the arithmetic mean that we may assert that the values of the constants a and b and of chronaxie do not change on falling asleep or awakening.

The results obtained with the subject V. were similar (4 recordings taken during daytime sleep, and one during nocturnal sleep). V. only fell asleep after taking 0.2 % sodium amytal, and her sleep was uneasy. As for A., the variations in excitability observed were associated with arm movements.

We examined the variations in excitability of the flexor digitorum sublimis muscle of our third subject, K., during nocturnal sleep. K. fell asleep readily, and slept soundly. We recorded a full strength–duration curve at 10-minute intervals. The excitability constants and the chronaxie were derived from the curves. Our observations showed that the fluctuations recorded were associated with arm movements, but not with falling asleep or awakening. Rises in the curves were, for this subject, due also to another factor, viz., to active contractions of the muscle, since they disappeared when the observer gently unclenched his fist. Chronaxie (derived from the curves, i.e., experimentally) varied very little.

It thus appears that our results are not in agreement with those reported by other authors, since in no case could we observe the increase in chronaxie during sleep, described by Bourguignon and Haldane, Kiselev and Maiorov, and others. We believe that the reason for this difference is that we recorded the full strength–time curves, and that we paid attention to the position of the arm during the experiments, and to the constancy of the pressure of the electrodes against the skin.

TABLE

Changes in the Values of the Constants a and b and of Chronaxie on Falling Asleep (Immediately, and After 30 Minutes) and on Waking (Subject A., Biceps Brachii Muscle)

Date	Falling asleep				Waking		Chronaxie		
	b		a		b	a	Falling asleep		Waking
	Immediately	after 30 min.	Immediately	after 30 min.	Immediately	Immediately	Immediately	after 30 min.	
11/15	+13	+13	+12	—	−37	−27	− 1	+20	+ 6
10/19	+ 3	+ 7	+17	+56	—	—	+13	+44	—
10/20	0	0	− 4	− 9	+ 7	− 2	− 4	− 8	− 8
10/20	− 3	0	−21	−16	0	− 5	−18	−17	− 4
10/23	− 8	− 8	− 5	− 6	—	—	0	+ 3	—
10/27	+10	+29	+13	+36	−2	0	+ 3	+ 5	+ 3
10/30	− 8	+ 4	+ 8	+ 3	—	—	+19	− 1	—
10/31	—	—	—	—	—	0	—	—	0
11/2	+ 3	—	+ 3	—	+ 8	0	+ 4	—	− 7
11/9	0	− 4	− 3	+ 6	−10	− 5	+10	+16	+ 6
11/10	− 5	0	0	0	—	—	+ 5	0	—
Mean	+0.5 ±2.24	+4.5 ±3.67	+2% ±1.127	+8.7 ±8.7	−5.66 ±6.71	−5.6 ±3.69	+3.1 ±1.017	+6.9 ±5.95	−0.57 ±2.24

We think it is possible that in some special cases sleep may be associated with changes in peripheral excitability. The results of our present research point, however, to the conclusion that no significant changes in the constants defining peripheral excitability take place during the forms of sleep studied by us.

LITERATURE CITED

[1] B. V. Andreev and F. P. Maiorov, Fiziol. Zhur. SSSR, 29, No. 3, 151-157 (1940).

[2] P. A. Kiselev and F. P. Maiorov, Fiziol. Zhur. SSSR, 27, 290-298 (1939).

[3] Ibid., 27, 299-308 (1939).

[4] I. I. Korotkov and N. A. Kryshova, Fifth Congress on Physiological Problems, pp. 44-46, Leningrad-Moscow, 1939.

[5] L. Lapicque, Newest Advances in the Knowledge of Nervous Mechanisms,* Leningrad, 1935.

[6] F. P. Maiorov, Arkhiv. Biol. Nauk SSSR 54, No. 1, 165-173 (1939).

[7] D. N. Nasonov and D. L. Rozental , Fiziol. Zhur. SSSR, 39, No. 4, 405-422 (1953).

[8] D. N. Nasonov and D. L. R zental , Fiziol. Zhur. SSSR, 42, No. 1, 78-87 (1956).

[9] M. I. Sandomirsku, Byull Eksptl. Biol. i Med., 1949.

[10] Ibid., Byull. Eksptl. Biol. i Med., 1949, No. 11, 341-343.

[11] A. L. Saper, Fiziol. Zhur. SSSR, 29, 135-143 (1940).

[12] M. M. Suslova, Fizioi. Zhur. SSSR, 29, 144-150 (1940).

[13] Yu. M Uflyand, Theory and Practice of Chronaximetry,* Leningrad, 1941.

[14] G. Bourguignon and J. B. Haldane, Compt. rend. Soc. biol., 1935, t. 107, pp. 1365-1366.

* In Russian.

EFFECT OF DURATION OF STIMULATING IMPULSES ON THE REACTION OF SKELETAL MUSCLE

N. N. Leonteva

From the Laboratory for the Physiology of Age (Director: Prof. A. N. Kabanov), Institute of Physical Training and School Hygiene (Director: A. A. Markosyan, Corresponding Member Acad. Pedagogic Sci. RSFSR), Acad. Pedagogic Sci. RSFSR, Moscow

(Received October 21, 1954. Presented by V. N. Chernigovsky, Member Acad. Med. Sci. USSR)

The generally accepted way of evaluating the functional possibilities of an excitable system is from a study of the way in which the excitation develops in the system. When we apply any form of stimulator to the study of the functional properties of a physiological substrate, the course of the excitation process developing will depend to a considerable extent on the properties of the stimulating agent. It is for this reason necessary in each case to make a precise evaluation of the stimulator used.

Until recent times, such evaluations were confined to a consideration of the frequency and the intensity of the stimulation, the significance of which for the response reaction of the excitable substrate was not appreciated in the 19th century. References may, however, be found in the literature to the circumstance that the nature of the response given by an organ is also significantly affected by the duration of the stimulating impulses [5, 11, 12, and 13].

The present paper is devoted to a study of the effect of varying the duration of stimulating impulses on the response to rhythmic stimulation.

EXPERIMENTAL METHODS AND RESULTS

Our experiments were carried out on adults and on children of the age groups 7-8, 11-12, and 14-15. Stimulating impulses of given durations were tested on each subject not less than 5-6 times. The source of rhythmic indirect stimulations of the muscles was an electric generator of square wave impulses. As the frequency of the impulses rises, for a given duration of stimulation, the kymograph records of tetanic contraction of muscle gradually rose to a maximum, after which the height of the contractions began to fall with increasing frequency; at very high frequencies the reaction was either totally abolished, or it took the form of "initial contraction." The object of our research was to find the frequencies at which the height of contraction first began to fall, and at which the response either abolished, or was of the "initial contraction" form. The former frequency gave us the threshold for the partial pessimum of the muscle, and the latter frequency the threshold for the full pessimum.

We stimulated the lower point of the median nerve of human subjects, and registered the movements of the thumb. The intensity of stimulation amounted to $1^1/_2$ times the tetanization threshold. The duration of the stimulating impulses was from 1 to 8 milliseconds.

We found that the lower the age of the subject, the sooner did increase in frequency of impulses, at constant duration, bring about a pessimal reaction. As the duration of the impulses was increased, however, both the partial and the full pessima were achieved at lower frequencies of stimulation, for all age groups (Table 1).

Increase in the period of stimulation had a greater effect on the partial and full pessima than did increase in the duration of the impulses. It follows that increase in the period of stimulation took place during the impulse and during the pause (Figure 1).

In passing from a shorter to a longer duration of impulse, the increase in the pause for which pessimal reaction of the muscle was found was not the same for children of different age groups: it was greater for 7-8-year olds than for older children. This observation may serve as an indirect indication of the lower lability of muscles of 7-8-year old children.

The same effect was found in adults when the duration of the impulses was raised to 8 milliseconds – the greater the duration of the impulse, the longer was the pause during which the muscle responded with a pessimal reaction (Figure 2).

TABLE 1

Mean Values of the Thresholds of Partial and Full Pessimum at Different Durations of Stimulation

Age	Duration of impulses, in msec.	Pessimum			
		partial		full	
		frequency	duration of pause, in msec.	frequency	duration of pause, in msec.
7—8 years	1	224	3.46	613	0.63
	2½	155	3.95	292	0.92
11—12 years	1	367	1.72	712	0.40
	2½	219	2.06	329	0.54
14—15 years	1	505	0.98	757	0.32
	2½	261	1.33	335	0.48
Adults	1	466	1.14	764	0.31
	2½	259	1.36	343	0.42
	4	125	4.00	—	—
	8	77	4.99	—	—

Thus the experiments in which duration was kept constant and frequency was varied showed clearly the dependence of the rate of onset of the pessimal reaction on the duration of the stimulating impulses.

Experiments in which we changed the duration of the impulses from one value to another, without interrupting stimulation or varying its frequency, showed that with relatively infrequent impulses the amplitude of the contractions increased when we switched over from shorter to longer durations. When the impulses were more frequent, increase in their duration had no effect on the amplitude of the contractions. At still higher frequencies, increase in duration led to a fall in the amplitude of the contractions.

Varying the frequency of the stimulation at constant duration showed that the greater duration of the impulses, the smaller was their frequency needed to give maximum amplitude of contraction. This evidently explains the strengthening of the motor reaction observed after increasing the duration of infrequent impulses. The data of the control experiments permitted two explanations of the absence of changes in the amplitudes of the contractions. In some cases both the durations applied, at the given frequency, gave an optimum effect, whereas in others the given frequency, in conjunction with the shorter duration, still gave a suboptimal effect, but at longer durations a pessimal one; both effects were of equal intensity.

The most pronounced changes in the amplitude of contraction were found in those cases in which the shorter duration gave an optimal effect, and the longer duration a marked pessimal one.

For example, application to one subject of stimulation of a frequency of 338 impulses per second, and a duration of 1 millisecond gave an optimal muscular reaction, whereas at the same frequency but with a duration of 2.5 msec. only a relatively small contraction resulted. For this reason at a frequency of 338 impulses per second the change in duration from 1 to 2.5 msec. resulted in a marked fall in the amplitude of the contraction. It follows that in order to demonstrate the effect of change in the duration of the stimulating impulse on the nature of the response reaction it is essential that the experiments should be performed using a wide range of frequencies, and not an arbitrarily selected one only.

We verified the results of our experiments by performing short experiments on 15 cats under anesthesia. We recorded the contractions of the anterior tibialis major muscle, in response to stimulation of the tibialis minor nerve, using a silver—silver chloride electrode. The indifferent electrode (a zinc sheet, area 6 sq. cm) was applied to the shaved skin in the lower abdominal region. The intensity of the stimulations was maximal, and the duration of the impulses was varied from 0.5 to 8 msec.

The animal experiments fully confirmed our findings, described above, with an even greater fall in the pessimum thresholds on increasing the duration of the stimulating impulses than for human subjects (Table 2).

The thresholds for full pessimum were determined only for durations of impulse of 1 to 8 msec. The values found were 175 and 74 impulses per second, respectively.

We also verified the dependence of the effect of stimulation on the duration of the impulses switching over from one duration to another without interrupting the experiment, without changing the frequency. As for humans, the change in amplitude of the tetanic contractions on switching over from a shorter to a longer duration of impulse depended on

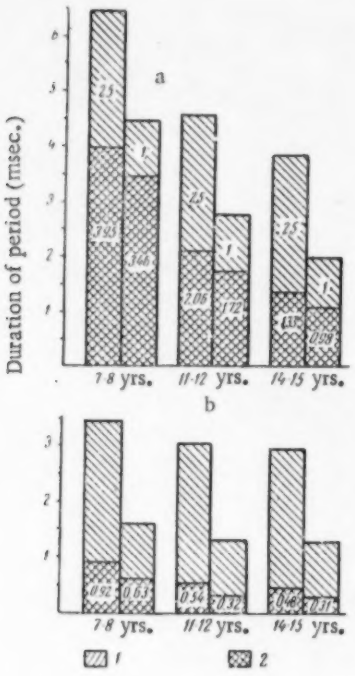


Fig. 1. Relation of the duration of the impulse to that of the pause at the frequency threshold of partial and full pessimum in children of different ages. a) Partial pessimum; b) full pessimum; 1) duration of impulses, in msec.; 2) duration of pauses, in msec

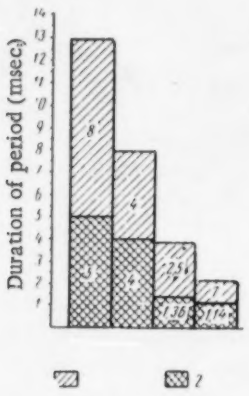


Fig. 2. Relation of the duration of the impulse to that of the pause at the frequency threshold of partial pessimum in adults. 1) Duration of impulse, in msec.; 2) duration of pause, in msec.

the frequency selected. Thus in one of the experiments (Figure 3), diminution in the amplitude of contraction first appeared with frequencies of 85 impulses per second, for duration of stimulating impulses of 1 msec. Increase in the duration of the impulses to 8 msec. led to a lowering of the partial pessimum threshold; a fall in amplitude was already evident at 50 impulses per second. Changing the duration of the impulses from 1 to 8 msec., at a frequency of stimulation of 30 impulses per second caused an increase in the motor reaction, while at a frequency of 50 impulses per second the amplitude of contractions fell, after an initial rise.

TABLE 2

Thresholds of Partial Frequency Pessimum in Cats

Duration in msec.		Pessimum threshold in imp./sec.	
impulses	pulse	mean value	range
0.5	6.91	135	104—150
1	8.71	103	75—132
4	12.67	60	50—75
8	13.74	46	10—55

Thus our observations on human subjects, and our experiments on cats, both with constant duration of impulses and variable frequency, and with constant frequency but variable duration, showed that the response reaction of the muscle is determined largely by the reciprocal relation of these two parameters. The greater the duration of the stimulating impulses, the smaller is the frequency necessary to produce a maximum response, and vice versa; the lower the frequency, the more does duration have to be increased to achieve a pessimal effect.

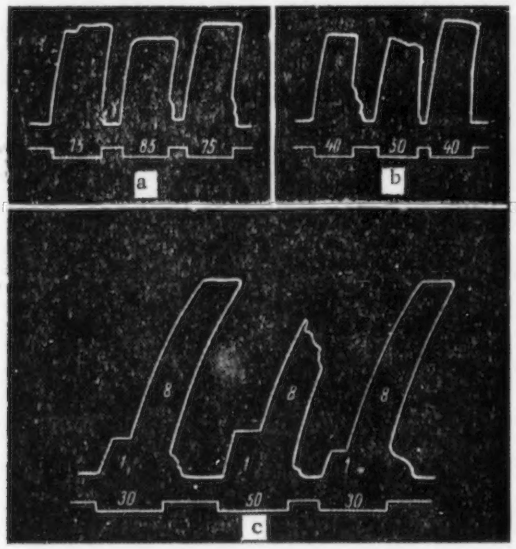


Fig. 3. Effect of varying the duration of impulses on the strength of contraction in response to stimulations of different frequency, in the cat. a) Partial pessimum threshold for duration of impulses of 1 msec.; b) the same, for durations of 8 msec.; c) switching over the durations from 1 to 8 msec., at frequencies of 30, 50, and again 30 imp./sec.
Explanation of tracings (from above down): muscle contractions, stimulation signals.

The more pronounced lowering of functional lability of the peripheral motor apparatus observed on raising the frequency or the duration of impulses in 7-8-year-old children is evidence of the lower resistance of neuromuscular elements to altering factors at this age.

It is of interest to consider this finding in conjunction with that of A. G. Ivanov-Smolensky [3], who showed that superlimiting inhibition occurs in the cerebral cortex more readily and frequently in school children of the younger age groups. This form of inhibition, according to I. P. Pavlov [10], has a protective effect, as it prevents the harmful action of excessively strong stimulation.

Other authors [8, 9] have shown that the same part is played by pessimal inhibition. It may be supposed that pessimal, or protective, inhibition arises in the neuromuscular apparatus more readily when the individual is younger [4].

These regular changes in the courses of the inhibitory process in children of different age groups are quite clearly shown in our experiments.

The lowering of the pessimum thresholds when the duration of stimulating impulses is increased may be explained, on the basis of N. E. Vvedensky's teachings on parabiosis, as a general reaction of living organisms to any factor, of either the internal or external environment [2].

The view that there is a close relationship between rhythmic stimulation and the parabiotic process is becoming increasingly widely held [1, 6, 7]. The excitation wave is regarded as a reversible parabiotic process. From this standpoint, the response reaction of the organ to the rhythmic stimulation depends on which particular phase of the reverse parabiotic reaction coincides with each successive impulse. If the second of a rhythmic series of stimulating impulses coincides with an inhibitory stage of the parabiotic process, a pessimal reaction results. Since each of the successive impulses intensifies the parabiotic process, maintaining it in the inhibtory stage, the pessimal reaction persists over the whole duration of stimulation. The longer the inhibitory phase of the parabiotic process is maintained, the smaller is the frequency which gives rise to a pessimal effect.

In our experiments, when we increased the duration of the stimulating impulses we thereby increased the duration of action of the dc cathode on the excitable substrate, and this, acting as a parabiotic agent, stopped the reverse parabiotic process.

LITERATURE CITED

[1] L. L. Vasilev, Advances in Reflexology and Physiology of the Nervous System,* 1929, Collection 3, pp. 31-42.

[2] N. E. Vvedensky, Excitation, Inhibition, and Narcosis,* St. Petersburg, 1901.

[3] A. G. Ivanov-Smolensky, Systematic Experimental Study of the Ontogenetic Development of the Dynamics of the Human Cerebral Cortex,* pp. 268-278, Moscow, 1940.

[4] A. N. Kabanov, Izvest. Akad. Pedagog. Nauk. RSFSR, 1954, No. 6, 124-126.

[5] M. V. Kirzon, Trans. Sci. Research Inst. Physiol., Leningrad Univ., 1934, No. 14, pp. 34-71.

[6] L. V. Latmanizova, Vvednensky's Laws of Electrical Activity of Excitable Units,* Leningrad, 1949.

[7] A. N. Magnitsky, Proceedings of the Congress in Honor of N. E. Vvedensky,* pp. 23-55. Moscow-Leningrad, 1949.

[8] A. N. Magnitsky and E. B. Perelman, Fiziol. Zhur. SSSR, 25, No. 3, 244-248 (1938).

[9] A. N. Magnitsky and V. D. Turbaba, Fiziol. Zhur. SSSR, 25, No. 3, 249-251 (1938).

[10] I. P. Pavlov, Complete Collected Works,* Vol. 3, Book 2. Moscow-Leningrad, 1951.

[11] V. D. Rosanova Fiziol. Zhur. SSSR, 30, No. 3, 346-356 (1941).

[12] Yu. M. Uflyand, Proceedings of the Congress in Honor of N. E. Vvedensky,* p. 73, Moscow-Leningrad, 1949.

[13] Yu. M. Uflyand and N. A. Shoshina, Fiziol. Zhur. SSSR, 23, 187-201 (1937).

ELECTROPHYSIOLOGICAL PHENOMENA IN NERVES DURING THE ACTION OF ANTIGENS ON SKIN RECEPTORS

A. N. Gordienko, V. I. Kiseleva, B. A. Saakov, and A. V. Leten

From the Chair of Pathol. Physiology (Director: Prof. A. N. Gordienko), Rostov Medical Institute

(Received March 16, 1956. Presented by V. N. Chernigovsky, Member Acad. Med. Sci. USSR)

With each year, more and more experimental evidence of the reflex mechanism of the action of antigens is being published, as well as of the development of processes of infection along these lines (A. D. Speransky, and co-workers, V. S. Galkin, G. V. Peshkovsky and co-workers, and others). Our laboratory has devoted many

* In Russian.

years to the investigation of the reflex mechanism of production of antibodies, and has found considerable evidence supporting its existence. We are, however, unable to find any publications dealing with the physiological analysis of the action of antigens on receptors, and with the path followed by the resulting excitation along the sensory channels. An antigen, in acting on a receptor, causes its excitation. The nature of this excitation may vary, according to the species of microorganism used for the purpose. The fact that specific antibodies are elaborated in response to different antigens is evidence of the specificity of the excitation evoked by the latter. An understanding of this effect may be assisted by an electrophysiological analysis of the processes arising in the receptors as a result of the action of antigens, and of the sensory conductors involved. With this object, we recorded the impulses proceeding through sensory channels from the stimulated receptors.

The electrophysiological study of sensory conductors during the action of antigens on receptors can also provide additional evidence in favor of the assumption regarding reflex elaboration of antibodies.

EXPERIMENTAL METHODS

The experiments were performed on dogs, under henexal anesthesia. The biopotentials were recorded from the auricular and the external saphenous nerves. For this purpose, the nerve was dissected free, and was split up, using a glass rod, into narrow strands, which were fastened to specially designed electrodes. We recorded the biopotentials from these nerves before and after intradermal injection of antigens and of physiological saline (control experiments) into different parts of the ear and the thigh. Amplification of the biocurrents was effected by means of the two-channel equipment 2-KUB-2, made by the Acad. Med. Sci. USSR Factory (1951), in a steel-lined chamber. The recordings were made on a moving strip of oscillographic paper, using a 9-channel oscillograph, made by Siemens and Halske (GDR) in 1951.

The biopotentials were taken from the auricular nerve before and after intradermal injection of typhoid, dysentery, or paratyphoid antigens (0.2 ml) into various parts of the ear. The recordings from the external saphenous nerve were also taken before and after injection of the antigens into the skin of the inner surface of the thigh. In the control experiments we injected the same volumes of physiological saline as of antigens.

EXPERIMENTAL AND DISCUSSION OF RESULTS

We performed 4 groups of experiments. In the first group (8 experiments) we recorded changes in the action potentials of the nerves in response to intradermal injection of typhoid antigen. The action potentials of the saphenous nerve were distinguished on the records by a background of low amplitude (up to 4-5 μv) waves of frequency over 50 cps, on which waves of a frequency of 6-7 cps and an amplitude of 10-12 μv could be clearly discerned. The background biopotentials of the auricular nerve consisted of waves of a frequency greater than 50 cps, interrupted by sporadic bursts of activity of a frequency of 18-20 cps and an amplitude of 5-6 μv.

In most cases, in response to injection of the typhoid antigen, there appeared a clearly discernable reaction, in the form of waves of a frequency of 5-6 per second, consisting of fast, low-voltage impulses, and of diphasic waves of an amplitude of 10-12 μv, which were replaced by regular rhythmic waves of an amplitude of up to 15 μv and a frequency 14-16 per second, and these in turn gave place to waves of frequency 18-20 per second and amplitude up to 25 μv. It should be noted that these waves appeared in the form of volleys, clearly evident on the oscillograms (Figure 1).

The above oscillogram illustrates the appearance of volleys, which were absent from the initial background record; the reaction was of short duration, since the recording had reverted to its original form 3 minutes after the injection.

The above findings are evidence that injection of antigen causes considerable change in the biopotentials of the nerves studied by us. It hence follows that typhoid antigen gives rise to excitation of skin receptors, recorded as action potential waves in the cutaneous nerves (Figure 1).

In the second group of experiments we investigated the changes in the electrophysiological activity of the same nerves following intradermal injection of paratyphoid B vaccine; this group consisted of 10 experiments. The initial background activity was identical with that described above. The most frequently encountered reaction to the antigen took the form of a continuous impulsation, composed of fast waves of amplitudes of 30-35 μv, which formed a background to volleys of spikes, of a duration of up to 10 seconds, and an amplitude of 90-100 μv (Figure 2).

The reaction did not appear immediately after introduction of antigen in all the experiments. In four cases injection of antigen was followed by a depression of all the rhythms, and this gave place, after 1-1½ minutes, to an activity of the type described above (Figure 3).

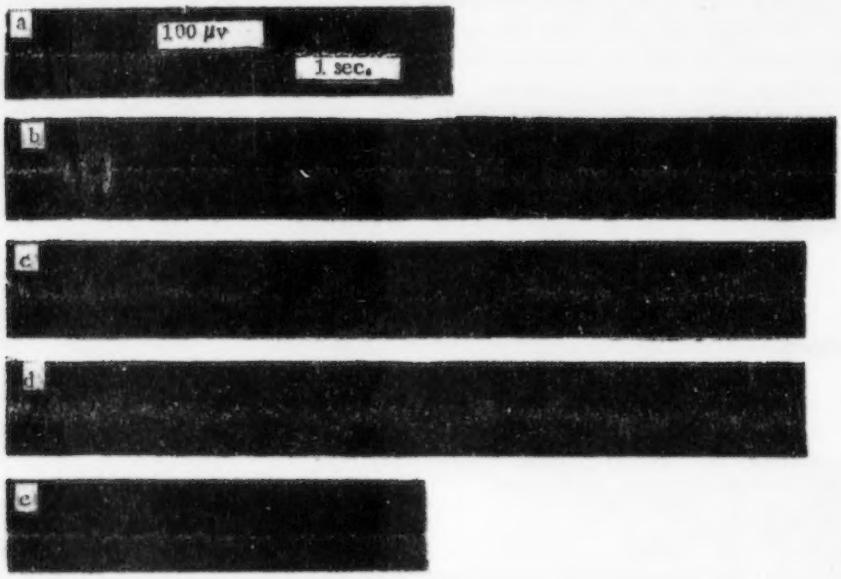


Fig. 1. Changes in bioelectrical activity of the auricular nerve in response to introduction of typhoid vaccine. a) Background; b) immediately after injection of vaccine; c) and d) 1 minute after; e) after three minutes.

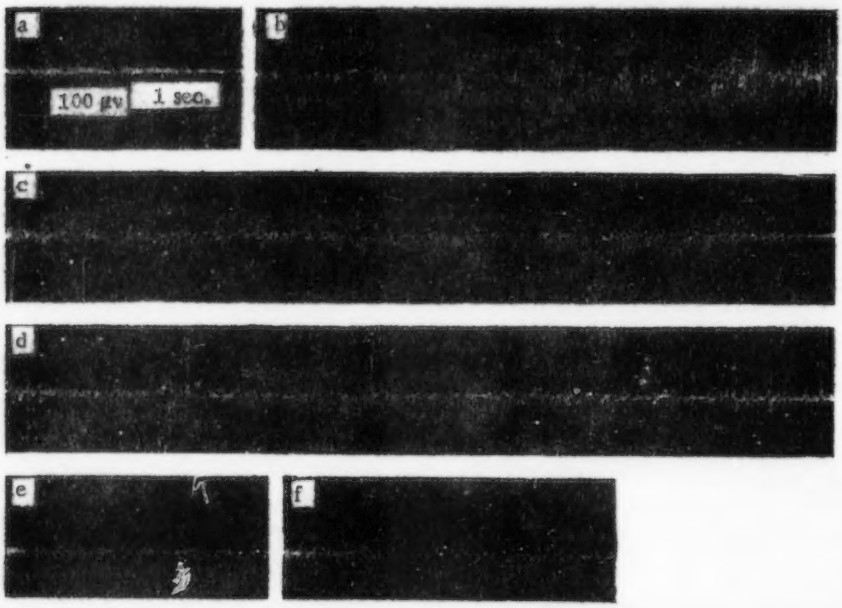


Fig. 2. Changes in bioelectric activity of the saphenous nerve in response to injection of paratyphoid B vaccine. a) Initial background activity; b), c), and d) immediately after injection of antigen; e) after 1 minute; f) after 2 minutes.

It is evident from Figure 3 that a depression of the initial biopotentials took place 20 seconds after injection of paratyphoid B antigen, followed after 1 minute by appearance of waves of a frequency of 15-18 periods per second, and of sporadic spike potentials, which are characteristically an effect of this antigen.

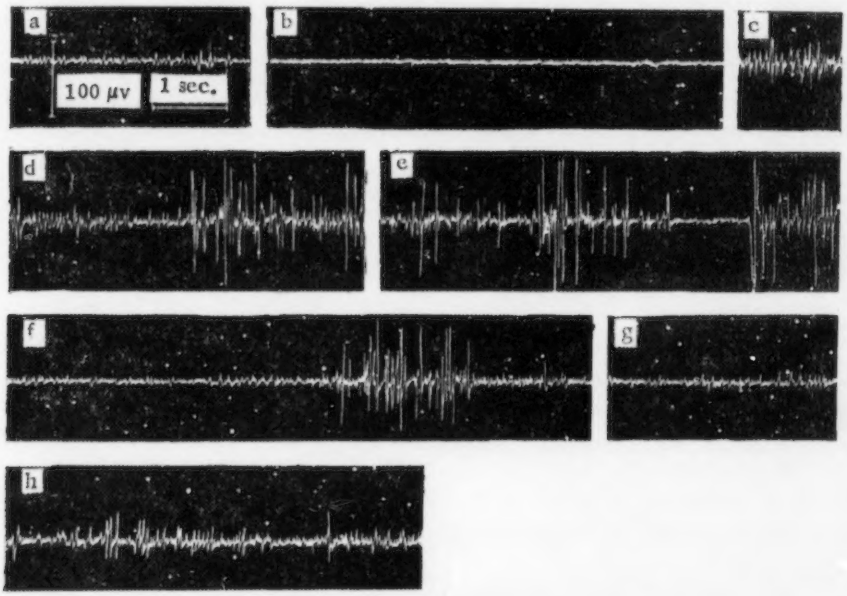


Fig. 3. Changes in bioelectrical activity of the saphenous nerve in response to injection of paratyphoid B vaccine. a) Initial background activity; b) immediately after injection of antigen; c) and d) after 1 minute; e) after 2 minutes; f) after 5 minutes; g) after 7 minutes; h) after 10 minutes.

In the third group of experiments we studied the effect on the biopotentials of the auricular and saphenous nerves of injection of dysentery antigen. Fourteen experiments were performed in this group, from which it appeared that the dysentery antigen is a powerful stimulator of skin receptors (this was expressed in the oscillograms by violent perturbations of the electrical oscillations registered from the nerves). We also found that the dysentery antigen could give rise to two types of reaction. The first type was characterized by appearance of the typical oscillations of biopotential immediately after introduction of the antigen, which, in the second type of reaction, was followed by a depression of electrical activity, lasting up to 1 minute, and then succeeded by Type 1 activity (Figure 4).

It is evident from Figure 4 that injection of dysentery antigen causes a violent reaction, expressed in the pronounced changes in frequency and amplitude of the action potentials of the nerves. In the first variant (Figure 4) the reaction supervened immediately after introduction of the antigen, while in the second variant this effect was preceded by depression of the initial rhythm. The potential waves seen when the reaction had developed were very similar to each other in both cases, which is, in our opinion, of significance. The reaction is characterized by the appearance of single and grouped discharges, of a frequency of 10-15 per second and an amplitude of 10-15 μv. After 1-3 minutes, but sometimes immediately, waves of a frequency of 8-10 per second, and of considerably greater amplitude, made their appearance.

As compared with the preceding groups, the reactions to introduction of dysentery antigen were of greater duration.

It thus appears that introduction of the above antigens results in stimulation of skin receptors, registered in our experiments from the changes in the action potentials of the sensory nerves. It might, however, be argued

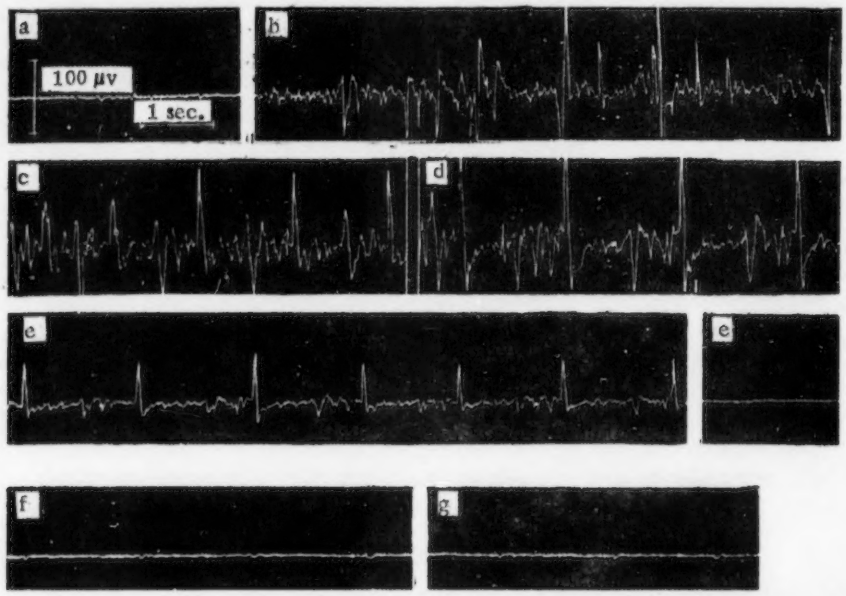


Fig. 4. Changes in bioelectric activity of the auricular nerve in response to injection of dysentery antigen. a) Initial background activity; b) after 20 seconds; c) after 1 minute; d) after 3 minutes; e) after 4 minutes; f) after 5 minutes; g) after 10 minutes.

that the potential changes recorded by us were merely an effect due to mechanical stimulation of the receptors by the prick of the needle or by the fluid introduced. In order to differentiate from such effects we performed 20 control experiments, in which physiological saline was injected, in the same amount as antigen. In these experiments we recorded the biopotentials from the same nerves, before or after injection of antigens. In some cases the saline was injected both before and after injection of antigen. In order to exclude any doubt regarding the possibility of the existence of a prolonged latent period, we performed experiments in which recordings of the electrical activity of the nerves were taken over a period of 15-20, or even 30 minutes after injection of saline. The results obtained showed that injection of physiological saline causes only insignificant changes in the oscillograms, and in some cases there was no change whatsoever in the background rhythm.

The reaction observed after injection of saline was characterized by appearance of fast waves of frequency up to 10 per second, and of fast waves, singly or in volleys, of amplitude 8-10 μv, during 1-2 minutes after injection.

OSCILLOGRAPHIC METHOD FOR MEASUREMENT OF FUNCTIONAL LABILITY AND REFRACTORY PHASE OF NERVES

P. I. Gulyaev

From the Laboratory for the Study of the Physiology of Higher Nervous Activity (Director: Dr. Biol. Sci. E. Sh. Zirapetyants), A. A. Ukhtomsky Physiological Institute, A. A. Zhdanov Leningrad State University (Director: Prof. N. V. Golikov)

(Received November 18, 1955. Presented by D. N. Nasonov, Member Acad. Med. Sci. USSR)

The measurement of the refractory phase of nerves or muscles is most often done by applying a single stimulation consisting of two impulses with a known time interval between them. The refractory phase can, however, also be measured under conditions of continous stimulation [3, 4, 7].

Advances in electronic technique have provided the possibility of producing two-stimulus stimulators, which are used for measurement of the refractory phase. Single- or double-beam oscillographs are generally used for this purpose [7]. We have devised a method for measurement of the refractory phase using a four-beam cathode oscillograph, with continuous or single stimulation.

Measurement of the refractory phase and lability of a nerve, applying continuous stimulation

By making use of automatic synchronization of processes on the screen of a cathode ray oscillograph, with periodic stimulation of the nerve by one of the stimuli, we obtain a stationary representation of the process on the screen of the oscillograph, which may be observed visually, or photographed [1]. If under these conditions of synchronization we apply the second stimulus to the nerve, at a given interval after the first one, then a second action potential may result, and the stationary image on the screen will represent two stimuli and two action potentials simultaneously. After the first action current, in response to the first stimulus, the nerve will be in a state of modified excitability, expressed as the absolute and relative refractory phases. The incidence, the extent, and the duration of these phases may be determined by applying the second stimulus at definite intervals of time. If the second stimulus is applied to the nerve at about 0.5-1 millisecond after the first, i.e., within the limits of the absolute refractory phase, the second action potential wave will not appear. It can be made to appear by gradually lengthening the interval between stimuli, and to pass through the various stages of the relative refractory phase, thus permitting the recording of the whole of the curve expressing restoration of excitability of the nerve.

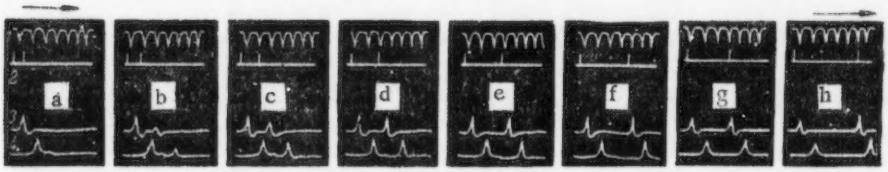


Fig. 1. Recording of the refractory phase of a nerve on the screen of a cathode ray oscillograph. a) to f): Oscillograms showing gradual development of the second action potential; g) oscillogram recorded with an adequate time interval between two series of stimulations. 1) Time marker, 500 cps; 2) exciting stimuli; 3) and 4) action potentials of the nerve.

For the measurement of the refractory phase by this method we used a 3-channel electronic stimulator of our own design, which allowed us to apply two stimuli to the nerve, the interval between which could be varied by turning a knob on the instrument. The stimulator generates three stimuli, the intervals between which can be varied within the limits 0-100 milliseconds. The amplitude, duration, and polarity of each stimulus can be varied independently of each other. The stimuli are generated either in the form of square waves or of that of a condenser discharge. The amplitude can be varied from 0 to 50 v, and the duration from 0.1 to 4 milliseconds. The

yield of the stimulator is symmetrical in relation to earth. In the experiments described below two stimuli were applied to the nerve from one and the same stimulating electrode, but our design of a 3-point stimulator would have permitted the application of the stimuli independently of each other, at different points along the nerve.

Figure 1 shows the whole process of origination of action potentials at different stages of the relative refractory phase, recorded on a 4-channel cathode ray oscillograph. Channel 1 shows time, in the form of periodic spikes, registered from a tuning-fork generator of frequency 500 cps; the interval between two spikes is therefore 2 milliseconds. Channel 2 registers the amplitude, form, and duration of the stimuli, and the interval between them. Channel 3 registers the diphasic action potential curve of the nerve, led-off from one pair of electrodes located at uninjured sections of the nerve. Channel 4 registers a monophasic action potential taken from a second pair of electrodes, of which one was placed in contact with a part of the nerve which had been killed by thermocauterization. It will be seen from the Figure that the action potentials of Channel 4 are displaced, with reference to those of Channel 3, by the time interval needed for propagation of the nerve impulse from one set of registering electrodes to the other. Knowing the distance between these electrodes, and the time interval between the stimuli, we can calculate the velocity of propagation of the nerve impulse.

Figure 1 shows successive stages of origination of a second action potential at different times of the relative refractory phase, applying square wave stimuli to the nerve. It is evident from oscillograms a to f that the second action potential appears and increases in amplitude as the time interval between the stimuli is lengthened.

The reason why we applied continuous stimulation was because two stimuli were applied to the nerve periodically (in the given case at a rate of $62\frac{1}{2}$ per second) for a previously determined period of time.

Figure 2 represents diagrammatically the entire process of measurement, recorded on a length of film moving at uniform speed. As is evident from Figure 2, not only the interval a, but also the intervals b and c, are of significance. In investigating the refractory phase under conditions of continuous stimulation, it is essential that the time interval between the first and the second stimulus should be adequate for restoration of full excitability of the nerve, before the next pair of stimuli is applied. Unless this condition is satisfied, and the first of the succeeding pair of impulses falls within the relative refractory period of the second impulse of the previous pair, the amplitude of the response to the second stimulus of the pair will be greater than to the first (Figure 1, g and h). In such a case, it is necessary to increase the time interval, i.e., to lower the frequency of repetition of pairs of stimuli, for example, from 62.5 to 50. The length of the interval will then be 20 milliseconds, and this would permit the study of the entire excitability cycle of normal nerves.

Fig. 2. Measurement of the refractory phases, recorded on a continuous moving strip of film. 1) Time marker; 2) stimulating impulses; 3) action potentials of the nerve; a), b), and c) intervals between stimulating impulses.

The procedure described above allows us to measure not only refractoriness, but also to assess the functional lability of the nerve. According to N. E. Vvedensky, lability is derived from the maximum frequency of stimulation which the nerve can reproduce without transformation, at a strength of current sufficient for the stimulation of all the fibers of the nerve. We also measured the lability of nerves under conditions of continuous stimulation, but instead of applying the maximum frequency we took that of the absolute refractory phase. From this value,

measured under conditions of tetanic contraction, we could calculate lability, according to N. E. Vvedensky, from the formula:

$$L = \frac{1000}{ar}$$

where L is lability, a is a constant, and r is the length of the absolute refractory phase, in milliseconds.

The procedure for deriving the lability coefficient from the length of the absolute refractory phase was first applied by A. A. Ukhtomsky [5, 6], and achieved in practice by P. O. Makarov [2]. The procedure devised by this author differs considerably from that of N. E. Vvedensky, since P. O. Makarov measured the length of the absolute refractory phase using single stimuli, whereas according to N. E. Vvedensky the lability coefficient is determined in the same way as in our method. Our method of calculation differs from that of A. A. Ukhtomsky in our introduction of the constant a, not equal to unity. The necessity of introducing this constant is illustrated by the following example. The length of the absolute refractory phase of the unfatigued nerve is usally about 1 millisecond. Hence if the value of the constant a were to be equal to 1, that of the lability coefficient would be 1000; according, however, to N. E. Vvedensky's measurements it is about 500. It follows that in the given case the constant a is equal to 2. The value of a should be determined by means of special experiments.

Measurement of refractory phase applying single stimuations

It is possible, taking a single time cycle of the cathode ray oscillograph, to measure the refractory phase, applying not continuous, but single stimulations of the nerve, as is done with the metronome ordinarily applied for the purpose. In such a case, the nerve is excited by two stimuli only, the second following the first at a known interval. The single stimulation method permits the recording on a film of the same effects as are recorded by the continuous stimulation method (shown in Figure 1), but is not adapted to continuous observation.

The advantages of the oscillographic over the metronome procedures reside in its greater objectivity and in its permitting of direct recording and prolonged visual observation of all the magnitudes concerned which cannot be achieved using a metronome equipment.

Automatic recording of the whole cycle of restoration of nerve excitability

The oscillographic method permits the observation and measurement of refractoriness at different points of the curve representing restoration of excitability, but does not, as usually applied, show the curve as a whole, in the form that the excitability curve is usually represented in textbooks. This can, however, be achieved in the following way. One stimulus is applied at close to zero time, while a series of second stimuli moves continuously along the screen, i.e., their incidence falls at different moments of the refractory phase. In consequence, the whole of the curve of recovery of excitability will be visible on the screen, as a line joining the summits of the action potential waves. An oscillogram of this type is shown in Figure 3. Channel 1 is used for the time marker

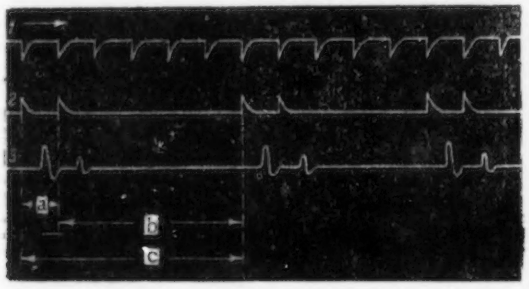


Fig. 3. Automatic recording on a cathode ray oscillograph screen of the whole of the curve expressing recovery of excitability. 1) Time marker; 2) action potentials of a nerve, arising at different stages of the relative refractory period.

(frequency 500cps). Channel 2 records the action potentials arising in response to stimulation. As is evident from the Figure, continuous stimulation of the nerve gives a picture on the screen in which the whole of the curve of recovery of excitability is visible, in the form of a line enclosing the action potential waves.

LITERATURE CITED

[1] P. I. Gulyaev and E. K. Zhukov, Methods of Electrophysiological Research,* Leningrad, 1948.

[2] P. O. Makarov, Transactions of the Leningrad Naturalists' Society, 54, No. 3, 319-350 (1935).

[3] D. I. Miminoshvili, Electrophysiological Characteristics of the Conducting Function of a Nerve Stump during the Regeneration Process, Thesis, Moscow, 1955.

[4] L. G. Trofimov, Fiziol. Zhur. SSSR, 35, No. 2, 182-198 (1949).

[5] A. A. Ukhtomsky, Collected Works,* Vol. 3, Leningrad, 1945.

[6] A. A. Ukhtomsky, Parabiosis,* Moscow, 1927.

[7] C. J. Dickinson, Electrophysiological Technique, London, 1950.

PROCEDURE FOR LEADING OFF ELECTRICAL POTENTIALS FROM AND FOR STIMULATING THE BASAL REGIONS OF THE BRAIN OF DOGS, UNDER CONDITIONS OF SERIES EXPERIMENTATION

G. Ya. Khvoles

From the Chair of Normal Physiology (Director: G. Ya. Khvoles), Karaganda Medical Institute (Director: Assistant Prof. P. M. Pospelov)

(Received November 28, 1955. Presented by Academician L. A. Orbeli)

A number of procedures are used in modern physiology for permanent leading off biopotentials from and for applying stimuli to, various parts of the brain. Of these procedures, the most useful has been that devised by A. B. Kogan [1], depending on the insertion of indwelling electrodes. By this procedure, action potentials can be led off from, and electrical stimulation can be applied to, the basal areas of the brain of dogs, using indwelling electrodes. This is a drawback of the method, since the electrode introduced into the brain injures conducting pathways and nuclear formations when being inserted into the desired locality, and may interfere with the normal physiological relations existing between different parts of the central nervous system. In this connection, we have devised a procedure for the direct leading off of bioelectric potentials from, and for the stimulation of, the basal areas of the brain under conditions of series experimentation in dogs, involving the designing of a Plexiglass screw-in electrode 10 mm long, with a diameter of the thread of 4 mm, and with 7 turns of the screw-thread.

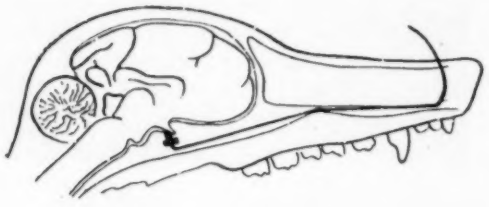


Fig. 1. Diagrammatic representation of the location of a basal screwed-in electrode, in the skull of a dog.

* In Russian.

A piece of silver or platinum wire 1.5 cm long and 1 mm in diameter, flattened at the end to a width of 2-3 mm, is inserted through a 1 mm hole in the threaded piece of Plexiglass.

The other end of the piece is provided with a square cap, to facilitate screwing in. An insulated multi-strand lead is soldered to the piece of wire inside the Plexiglass, and is led out through the nasal passage to the skin surface of the bridge of the nose, at the boundary between its bony and cartilaginous parts (Figure 1). For short-term experiments, plated wood-screws may be used instead of the above arrangement, the dimensions being the same.

The operation of insertion of the electrodes is conducted under general hexenal anesthesia. A 10% solution of hexenal is injected into the saphenous vein, at a dosage level of 0.2-0.5 ml/kg body weight. The dog is tied down on its back. The assistant raises the lower jaw and depresses the upper one, by means of two napkins. The tongue is caught in a pair of tongue-retractors, and held out of the mouth. The soft palate is swabbed with alcohol, and split longitudinally, from the hard palate to the uvula. The edges of the wound are retracted with two pairs of hemostatic clamps, thus widely exposing the nasopharynx. A cruciform incision is made into the mucous membrane and periosteum of the palatal bone, using a sharp lancet, and the tissues are retracted with a raspatory, exposing the bone. A hole is bored with a drill through the palatal bone, at its junction with the sphenoid. The anterior hypothalamus is located here. A metal sleeve is fitted to the drill, so as to act as a guard against penetration of the drill into the brain substance. A screw-tap is inserted into the opening in the bone, and a thread is cut to accommodate that of the electrode. When the screw-tap has penetrated to the meninges, the surrounding tissues and the bony canal are treated with penicillin solution, and the electrode, previously sterilized by boiling in water, immersion in alcohol, and treatment with penicillin, is screwed in. A steel wire ending in a narrow loop is then inserted through a nostril into the nasopharynx. Catching the electrode lead in the loop, we pull it out through the nostril. A passage from the nasal cavity to the exterior surface of the bridge of the nose is pierced by means of a narrow lancet or a blade of a pair of ophthalmological scissors, at the junction of the bony and the cartilaginous parts of the nose. The tip of the lancet is seized in a small hemostatic clamp, which is pulled down through the opening made in the nose to the exterior. The knife is then released, and the electrode lead is seized in the jaws of the clamp, drawn up through the passage to the exterior, and sutured in position on the bridge of the nose. The wound in the soft palate is closed, using three silk stitches. The postoperational course is usually uncomplicated, and the dog may be taken for experimentation within 7-10 days. A basal electrode implanted in this way remains very firmly attached to the bone. We have used similar screw electrodes for recording the electrical potentials of the cerebral hemispheres, and we are thus able to study cortical-subcortical interrelations.

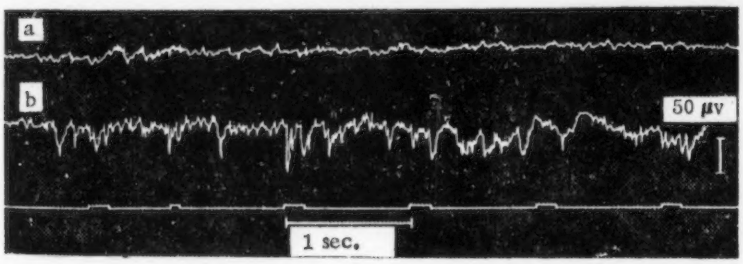


Fig. 2. Tracings of biopotentials recorded from the anterobasilar areas of a dog's brain, by the procedure described. a) Normal tracing, before applying a therapeutic mudpack; b) after the application.

Figure 2 presents recordings of biopotentials from the anterior hypothalamic region of a dog's brain.

LITERATURE CITED

[1] A. B. Kogan, Technique of Implantation of Indwelling Electrodes for the Recording of Potentials from, and the Stimulation of, the Brain, Moscow, 1952.

• In Russian.

BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE IN ENGLISH TRANSLATION

Supplement to No. 1

1957

TABLE OF CONTENTS

	Page	Russ. Page
PHYSIOLOGY AND PATHOLOGY OF THE CARDIOVASCULAR SYSTEM		
EXPERIMENTAL INVESTIGATIONS		
1. Study of Coronary Blood Flow in Unanesthetized Dogs. M. E. Marshak and G. N. Aronova	1	3
2. Reflex Effects on Vascular Tonus from Cardiac Mechanoreceptors. N. I. Lukshina and A. M. Ugolev	3	6
3. Significance of the Reflex from the Superior Vena Cava in the Regulation of Blood and Lymph Circulation. V. V. Petrovsky	8	10
4. Influence of Splenic Interoceptors on Blood Pressure and Respiration in Ontogenesis. L. A. Palgova	10	13
5. Experimental Pituitrin Hypertension and Coronary Insufficiency in Monkeys. A. A. Belous and G. O. Magakyan	14	17
6. Regulation of Disordered Rhythm of the Heart by Means of Procaine Block of Extracardial Nervous Formations, and of other Agents Affecting the Nervous System. B. M. Fedorov	18	21
7. Functional Disturbances Following Massive Infusions of Physiological Saline. G. L. Lyuban	22	26
8. Rate of Elimination of Congo Red from the Blood Stream of Decerebrate Dogs. S. A. Chesnokova	25	30
9. Reflex Changes in the Intracardial Nervous Formations Encountered in Focal Experimental Myocarditis. V. S. Livshits and L. N. Lebedeva	27	32
10. Alterations in Phagocytic Activity of the Leucocytes of Human Blood During Anesthesia. Z. E. Matusis and V. V. Sherstobitova	31	36
11. Effect of Barbiturates on the Differential Cell Count of Peripheral Blood of Normal Animals and in Experimental Anemia. M. F. Runova	33	38
12. Udder Reception in Cows and Goats, and the Composition of the Blood. O. P. Belygina	36	42
13. Blood Changes During Physiological Sleep. M. I. Ulyanov	39	45
NEW METHODS		
14. Technique of Determining the Rate of Propagation of the Pulse Wave in Humans and Animals. V. E. Busygin and Yu. G. Nefedov	42	48
15. Method for Determining the Circulating Blood Volume, for Experimental Purposes. Yu. G. Nefedov	44	50
16. Photographic Method of Study of the Vascular Permeability of the Hemato-Ophthalmic Barrier and the Skin. N. I. Arlashchenko	46	52
17. Interpretation of Human Plethysmographic Data. E. N. Kondratev	48	54

	Page	Russ. Page
18. Some Remarks Relating to the Technique of Interpretation of Plethysmographic Data. L. G. Okhnyanskaya.	52	57
19. Recording of Electrograms of Workmen During Industrial Work. L. A. Vodolazsky.	54	59
20. Instrument for Narrowing the Lumen of the Aorta, in Short Experiments. A. Kh. Khaidarov.	56	61
21. Measurement of Height of Electrocardiogram Waves. D. P. Kochetov and B. S. Tikhonov.	58	63
22. New Model of Experimental Hypertension Produced by Ischemization of the Spleen. F. A. Morokhov.	61	64
23. Technique of Experimental Production of a Venous Thrombus. Z. D. Bleksmit and M. A. Kotovshchikova.	63	67
PATHOLOGY AND PHYSIOLOGY OF DIGESTION		
EXPERIMENTAL INVESTIGATIONS		
24. Effect of Ablation of the Cerebral Cortex on the Secretory Activity of the Salivary Glands of Dogs. T. A. Maltseva.	63	68
25. Effect of a Circumscribed Focus of Inflammation in the Buccal Cavity on the "Pilocarpine" Secretion of the Parotid Glands. A. P. Savinskaya.	67	71
26. Role of the Functional State of the Alimentary Center in the Motor-Evacuatory Activity of the Stomach of Dogs. I. V. Malkiman and M. A. Vasilevsky.	70	74
27. Effect of Stimulation of the Hypothalamus on the Motor-Secretory Activity of the Empty Stomach, in Extensive Experiments on Dogs. A. F. Kosenko.	74	79
28. Effect of Natural and Drug-Induced Sleep on Gastric Secretion in Dogs. N. Sh. Amirov.	78	83
29. Inhibitory Action of Ribonuclease on Secretion of Hydrochloric Acid by Surviving Frog's Gastric Mucosa. Ya. A. Epshtein.	81	86
30. Effect of Intravenous Injection of Heterologous Serum on Gastric Secretion. N. E. Dukler.	83	88
31. Effects of Changes in the Functional State of the Cerebral Cortex on Secretion of Bile. I. T. Dzheliev.	87	92
32. Bile Secretion after Partial Resection of the Liver of Dogs with an Exteriorized Common Bile Duct. B. P. Solopaev.	90	95
33. Effect of the Drug Tetamon-I on the Bile Secreting Function of the Liver. N. P. Skakun and A. Ya. Sereda.	94	99
34. Pancreatic Secretion in Response to Different Amounts and Concentrations of Hydrochloric Acid Solutions. O. B. Sobieva.	97	100
35. Secretion of Intestinal Enzymes by Isolated Intestinal Segments at Different Intervals after "Denervation." L. S. Fomina.	100	105
36. Effect of Ascarid Toxin on Excitability of Intestinal Chemoceptors. I. A. Edelshtein.	106	111
37. Effect of the Thyroid Hormone on the Evacuatory Function of the Stomach. S. G. Genes and N. G. Lesnoi.	108	113

TABLE OF CONTENTS (continued)

	Page	Russ. Page
NEW METHODS		
38. An Intestinal Fistula Tube. A. N. Bakuradze.	113	117
ELECTROPHYSIOLOGY AND NEUROMUSCULAR PHYSIOLOGY		
EXPERIMENTAL INVESTIGATIONS		
39. Some Possible Forms of Intensification of Slow Waves in the Electroencephalogram. N. P. Bekhtereva.	114	119
40. Effect of Stimulation of Rectal Receptors of Dogs on the Electrical Activity of the Brain. N. N. Lazuko.	118	123
41. Effect of Progesterone, Folliculin and Castration on the Electrogencephalogram of Female Rabbits. G. F. Khrustaleva.	121	126
42. Effect of Stimulation of the Hypothalamic Region on Reflex Fluctuations of Skin Potentials of Frogs. I. V. Vvedenskaya.	124	129
43. Determination of Excitability of Human Muscles. D. L. Rozental and V. N. Filippova. . . .	127	132
44. Excitability of Human Muscles During Sleep and Wakefulness. D. L. Rozental and V. N. Filippova.	132	138
45. Effect of Duration of Stimulating Impulses on the Reaction of Skeletal Muscle. N. N. Leonteva.	136	142
46. Electrophysiological Phenomena in Nerves During the Action of Antigens on Skin Receptors. A. N. Gordienko, V. I. Kiseleva, B. A. Saakov, and A. V. Leten.	140	147
NEW METHODS		
47. Oscillographic Method for Measurement of Functional Lability and Refractory Phase of Nerves. P. I. Gulyaev.	145	151
48. Procedure for Leading off Electrical Potentials from and for Stimulating the Basal Regions of, the Brain of Dogs, Under Conditions of Series Experimentation. G. Ya. Khvoles. . . .	148	155

ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO.	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci. Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

THE A·I·B·S bulletin

Official Publication Of

NEWS AND VIEWS IN THE BIOLOGICAL SCIENCES

THE AMERICAN INSTITUTE OF BIOLOGICAL SCIENCES
2000 P Street, N.W., Washington 6, D.C.
ADams 4-5573

If you are a biologist....

If you are interested in the biological sciences....

The AIBS BULLETIN is a "must".

This magazine offers news and information important to every biologist. It carries book reviews, brief and pertinent, on all new publications in the field. Feature articles deal with all manner of general and controversial topics in biology. The advertisers are specialists in products needed by every teacher and research worker. The August issue is a special General Program for the annual meeting of biological societies that the Institute sponsors.

The AIBS BULLETIN may be obtained in any one of the following ways.

1. Through your membership in one of the nineteen Member Societies of the Institute, you automatically receive the BULLETIN free of charge, as part of your membership dues.
2. If you belong to one of the eighteen Affiliate Societies of the Institute, you may subscribe to the BULLETIN for $1.00 per year.
3. Regular subscription prices for non-members are: U.S.A., $2.00 per year; Canada, South and Central America, $2.50; all other foreign, $3.00.
4. Various categories of Individual Membership are offered by A.I.B.S.: Associate, $1.00; Professional, $3.00; Supporting, $10.00; and Life, $100.00. A subscription to the BULLETIN accompanies each type of membership.

FIVE ISSUES YEARLY

AMERICAN INSTITUTE OF BIOLOGICAL SCIENCES
2000 P Street, N.W., Washington 6, D. C.

THOUSANDS OF JOURNALS

throughout the world are publishing significant research papers in

Experimental Biology and Medicine

any one of which might be of vital interest to you

How do you keep posted on current advances in your field? No individual possibly could read it all in the original even if the journals were available to him. *Biological Abstracts* does this arduous task for you. Currently publishing more than 40,000 abstracts of research papers in biology, it plans gradually to expand its coverage to 80,000 within the next ten years in order to keep pace with the ever-increasing output of biological literature.

Biological Abstracts, a cooperative, non-profit undertaking, is published by biologists for biologists in a complete edition covering all fields of biology, and five low-priced sectional editions that are specially designed for individual use. Two of these sectional editions have particular value for those working in Experimental Biology and Medicine:

SECTION B—each month 1,200 to 1,500 abstracts of research papers in Animal Physiology, Pharmacology, Morphology and Pathology, exclusive of Microbiology, are published.

SECTION C—each month some 450 to 550 research papers in Microbiology, including General Bacteriology, Medical Bacteriology, Viruses, Pathogenic Fungi, Chemotherapy, Immunology, Public Health, Industrial Microbiology, and Parasitology are abstracted.

Individuals are allowed a discount of 50% off the list prices of the sectional editions if the subscriptions are for their personal use.

Sample copies and complete information will be sent upon request.

BIOLOGICAL ABSTRACTS, University of Pennsylvania
3815 Walnut Street, Philadelphia 4, Pa.